Long-term discordant fluctuation of chronic stress and immune biomarkers in children and adolescents affected by the Great East Japan earthquake

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Abstract Although reconstruction from the 2011 Great East Japan earthquake and tsunami is proceeding, the environment of children living in the disaster area has not yet recovered. Anxiety among such children may have adverse effects on their future mental and physical health. The purpose of this study was to examine fluctuations in chronic stress and immune conditions in children and adolescents living in the disaster area. The participants were elementary and junior high school students living in the Pacific coastal area of northern Japan. This serial cross-sectional study performed five surveys in September 2011 (6 months, n = 391), March 2012 (1 year, n = 394), March 2013 (2 years, n = 281), March 2014 (3 years, n = 332), and March 2015 (4 years, n = 313). Stimulated whole saliva samples were collected, and saliva flow, secretory immunoglobulin A concentration, secretory immunoglobulin A secretion rate, and cortisol concentration were determined. Cortisol concentration significantly decreased from 6 months to year 2 of the study period, but increased between year 2 and year 4. Secretory immunoglobulin A concentration significantly increased between year 1 and year 4 compared to the first 6 months. Gender differences were observed in saliva flow and secretory immunoglobulin A concentration, and significant differences between elementary and junior high school students were observed in cortisol concentration. Therefore, fluctuation of cortisol concentration as a chronic stress biomarker and an increase in SIgA as an immune biomarker were observed during the 4 years after the disaster, but the changes in the two biomarkers did not correspond.

Keywords: disasters, mental health, cortisol, secretory IgA, Japanese children and adolescents

Introduction

The Great East Japan earthquake and tsunami that occurred in March 2011 caused serious damage to the lives and the environment of the people who lived in the stricken area. Recovery and reconstruction from the disaster are not yet complete⁷. It was previously reported that changes in the living environment caused by the disaster have had an enormous influence on the mental and physical health of the people who live in the area²⁻⁴. Chronic biological and behavioral stress causes obesity and metabolic abnormalities, resulting in diabetes and metabolic syndrome and increased risks of heart disease and other acute clinical events⁷. It is necessary to understand the mental and physical health of people after the disaster and to take appropriate measures for the maintenance and improvement of their future health. In addition, these matters and coping strategies are also important concerns for the research field of health and physical science.

Cortisol, the terminal hormone of the hypothalamic-pituitary-adrenocortical axis (HPAA), has a sensitive reaction to acute and chronic stimuli, including psychological stress, physical activity, and exercise. The production of cortisol is altered by the intensity of the stimuli. Cortisol functions in the control of blood pressure, glucose metabolism and gluconeogenesis, protein and lipid metabolism, and immunosuppression. It is suggested that a long-term elevation of blood cortisol concentration is induced in atrophy of the hippocampus⁷⁻⁸, indicating a relationship between post-traumatic stress disorder (PTSD) and cortisol⁹. For example, high morning concentrations of serum interleukin-6 and high evening concentrations of salivary cortisol are related to later PTSD development⁷⁻¹⁰. Therefore, there is a possibility that changes in these biomarkers are predictive factors of PTSD¹¹. Salivary secretory immunoglobulin A (SIgA) is a major factor of the mucosal immune system and plays an important role in the first-line of defense of the immune...
development. Decline or disappearance of SlgA secretion increases the possibility of infection by viruses and microorganisms, and indicates the danger of disease.

With regard to the influence of the experience of a large-scale natural disaster on psychosomatic health, symptoms of PTSD were observed in children and adolescents affected by the 2011 earthquake and tsunami, and these symptoms have been gradually reduced over time since the disaster. Thus, although a significant amount of variation is expected in chronic stress indicators such as cortisol and SlgA, there are few reports of long-term changes in chronic stress biomarkers over several years among children and adolescents. In addition to the effects of acute stress stimulation on the HPAA and the immune system in children and adolescents, there are many reports of the impact of acute stress on the mind and body.

The purpose of this study was to consider the long-term fluctuations of chronic stress and immune biomarkers and the relationship between cortisol and SlgA in elementary and junior high school students who were living in the disaster area of the 2011 Great East Japan earthquake and tsunami.

Materials and Methods

Participants. The participants in this study were elementary school students in the 4th to 6th grades and junior high school students in the 7th to 9th grades. This serial cross-sectional study included five inventory surveys in September 2011 (6 months after the disaster), March 2012 (1 year after the disaster), March 2013 (2 years after the disaster), March 2014 (3 years after the disaster), and March 2015 (4 years after the disaster) in Onagawa, Miyagi Prefecture, in the Pacific coastal area of northern Japan. The study was approved by the Research Ethics Committee of Tohoku Gakuin University Graduate School, Division of Human Informatics (No. R2011.0001). All subjects and their parents or legal guardians provided written informed consent.

Saliva collection. To determine saliva flow (SF), saliva SlgA concentration (SlgA-C), and cortisol concentration (COR), stimulated whole saliva samples were collected between 3 pm and 4 pm to avoid diurnal fluctuations, using a “Sali-Kids” saliva sampling tube (Sarstedt, Nümbrecht, Germany). In brief, before collecting each sample, the participant rinsed the inside of the mouth with tap water once, and then rested in a sitting position for 5 min. Next, each participant swallowed his or her saliva to dry the inside of the mouth and chewed an absorber of polypropylene/polyethylene polymer in order to collect the newly secreted saliva at a frequency of 60 times/min for 1 min. Then, the participant placed the absorber into a plastic tube, and saliva was extracted from the absorber by centrifugation at 1600 g for 5 min. After measurement of the sample volume, the saliva samples were frozen at −80°C for later analysis.

Salivary cortisol and SlgA measurements. Salivary COR was determined by use of a commercial salivary cortisol enzyme immunoassay kit (1-3002, Salimetrics, Carlsbad, CA, USA) following the manufacturer’s instructions. SlgA-C was determined by enzyme-linked immunosorbent assay (ELISA) as described previously.

To avoid interassay variability, all samples from each subject were assayed on the same microtiter plate. The interassay coefficient of variation was <5% for both assays. Saliva SlgA data were expressed as SlgA-C (µg/ml) or the SlgA secretion rate (SlgA-SR) (µg/min). SlgA-SR was calculated as the product of SF (ml/min) and SlgA-C.

Statistical analysis. Data are reported as the mean ± standard deviation unless otherwise stated. Prior to statistical analysis, assumptions of constant variance and normality of the data were assessed. If an assumption violation was detected, the data were stabilized by transformation prior to further statistical analysis. Salivary cortisol and SlgA measurements were compared using generalized multivariate analysis of variance (ANOVA). When significant effects were detected (p < 0.05), the location of significance was determined using separate Student’s t-tests with Tukey’s test for multiple comparisons. To investigate the relevance of the measurements, partial correlation analysis was performed using gender, school grade, and investigation time as adjustment variables. All statistical analyses were performed with SPSS version 21 for Windows (Chicago, IL, USA).

Results

Fluctuation of salivary biomarkers of chronic stress and mucosal immune response. Table 1 gives the details of the participants. To evaluate the state of chronic stress and mucosal immune function of children and adolescents living in the disaster area, fluctuations of salivary cortisol and SlgA measurements were observed in five surveys over 4 years. ANOVA showed significant changes in SF \( F(4, 1732) = 5.18, p < 0.001, \text{Fig. 1A} \), SlgA-C \( F(4, 1732) = 9.68, p < 0.001, \text{Fig. 1B} \), SlgA-SR \( F(4, 1732) = 8.16, p < 0.001, \text{Fig. 1C} \), and COR \( F(4, 1732) = 29.83, p < 0.001, \text{Fig. 1D} \). Tukey’s tests revealed that SlgA-C was...
Chronic stress and immune response after the disaster significantly increased at year 1 to year 4 compared to at 6 months. In contrast, salivary COR was significantly decreased at years 1 to 3 compared to at 6 months. Both SF and SIgA-SR showed a fluctuation with a similar pattern. Interestingly, COR was significantly increased at years 3 and 4 compared to at year 2.

Comparison of the salivary biomarkers between boys and girls. We examined the effect of gender differences in fluctuation of salivary biomarkers during the investigation period using two-way ANOVA and post hoc Tukey’s test. There were significant differences in SF \( F(1, 1735) = 20.15, p < 0.001, \text{Fig. 2A} \) and SlgA-C \( F(1, 1735) = 27.53, p < 0.001, \text{Fig. 2B} \). There were no significant differences in SIgA-SR \( F(1, 1735) = 0.17, p = 0.676, \text{Fig. 2C} \) or COR \( F(1, 1735) = 3.77, p = 0.052, \text{Fig. 2D} \). However, significant [Period][Gender] interaction effects were found for COR \( F(4, 1732) = 2.43, p < 0.05, \text{Fig. 2D} \), but not for SF, SlgA-C, or SlgA-SR.

| Table 1. Demographic characteristics. |
|---------------------------------------|
|                                      |
| All subjects (N)                     |
| 403 403 286 332 313 1737             |
| Girls (%)                            |
| 49.6 51.1 51.4 48.5 51.4 50.4        |
| ESa (N)                              |
| 199 199 169 153 132 852              |
| Girls (%)                            |
| 50.8 52.8 50.9 49.0 47.0 50.4        |
| JHSb (N)                             |
| 204 204 117 179 181 885              |
| Girls (%)                            |
| 48.5 49.5 52.1 48.0 54.7 50.4        |

\(^a\)4th to 6th grade elementary school students, \(^b\)7th to 9th grade junior high school students.

Fig. 1 Fluctuation of chronic stress and mucosal immune biomarkers of children and adolescents after the disaster. Saliva samples obtained from the subjects were measured for SF (ml/min) (A), SlgA-C (µg/ml) (B), SlgA-SR (µg/min) (C) and COR (µg/dL) (D). Data were expressed as mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001.
Comparison of the salivary biomarkers between elementary and junior high school students. To examine the effects of differences in grades on fluctuation of salivary biomarkers during the investigation period, the subjects were divided into two grade-classified groups (elementary school students [ES]: 4th to 6th grades, and junior high school students [JHS]: 7th to 9th grades), and the differences were analyzed by two-way ANOVA and post hoc Tukey’s test. There were significant differences in COR \[F(1, 1735) = 137.30, p < 0.001, \text{Fig. 3D}\], but there were no significant differences in SF, SIgA-C, or SIgA-SR. In addition, significant [Period]*[Grade] interaction effects were found in SF \[F(4, 1732) = 4.09, p < 0.01, \text{Fig. 3A}\], SIgA-C \[F(4, 1732) = 5.32, p < 0.001, \text{Fig. 3B}\], SIgA-SR \[F(4, 1732) = 4.85, p < 0.001, \text{Fig. 3C}\], and COR \[F(4, 1732) = 7.05, p < 0.001, \text{Fig. 3D}\].

Correlation among salivary chronic stress and immune biomarkers. We carried out partial correlation analysis using gender, grade, and investigation time as adjustment variables to consider the relation between COR and the mucosal immune biomarkers, such as SF, SIgA-C, and SIgA-SR. There wasn’t any significant correlation between COR and SIgA-C, but there was significant, though weak positive correlation between COR and SF or SIgA-SR for all, gender- and school grade-divided participants \(p < 0.05, \text{Table 2}\). When correlation within an investigation period was considered, there was weak, but positive correlation between COR and SF or SIgA-SR at 6 months. Moreover, there was significant, but weak positive correlation between COR and SIgA-C at year 3 \(p < 0.05, \text{Table 2}\).

Discussion
In this study, we examined consecutive changes over 4 years in salivary chronic stress and immune biomarkers among elementary and junior high school students who were living in the coastal areas affected by the 2011 Great East Japan earthquake and tsunami. Significant fluctuations were observed in both stress and immune biomarkers over a 4-year period following the disaster. Moreover, significant differences were observed between boys and girls in the immune biomarkers and between elementary (ES) and junior high school (JHS) students in the stress biomarker.

Among the chronic stress and immune biomarkers, the production of COR significantly decreased from 6 months to 2 years after the disaster, but it showed a significant increase thereafter; whereas SIgA-C increased significantly from 6 months after the disaster (Fig. 1). PTSD symptoms have been reported in children and adolescents living in the affected areas, which gradually recovered over time after the disaster.\textsuperscript{20,21,25} In addition, the association be-
between PTSD and cortisol has been pointed out in several previous studies. Therefore, it is considered that COR increased as a result of acute stress resulting from the disaster experience and gradually decreased as the influence of acute stress gradually declined over time. There have been no reports of long-term fluctuation of SIgA indexes of affected subjects, but stressful life events have been reported to lower SIgA levels chronically. It is thought that the increase in the production of COR causes a decrease in the number of B cells, and consequently the production of SIgA slows down. Moreover, it has been shown that a chronic increase in the production of cortisol

Table 2. Correlation between COR and each SIgA factor.

| COR of | SF  | SIgA-C | SIgA-SR |
|--------|-----|--------|---------|
| All subjects | 0.086 *** | 0.020 | 0.088 *** |
| Boys | 0.092 ** | 0.034 | 0.085 * |
| Girls | 0.077 * | 0.012 | 0.090 ** |
| ES | 0.116 *** | 0.030 | 0.119 *** |
| JHSb | 0.077 * | 0.011 | 0.071 * |
| 6m | 0.172 *** | 0.059 | 0.207 * |
| 1y | 0.089 | -0.021 | 0.063 |
| 2y | 0.043 | 0.016 | 0.053 |
| 3y | 0.022 | 0.112 * | 0.085 |
| 4y | 0.039 | 0.013 | 0.045 |

Data were expressed by correlation coefficient. *p < 0.05, **p < 0.01, ***p < 0.001.

a4th to 6th grade elementary school students, b7th to 9th grade junior high school students.

Fig. 3 Group differences according to grade in the fluctuation of chronic stress and mucosal immune biomarkers after the disaster. Saliva samples were measured for SF (ml/min) (A), SIgA-C (μg/ml) (B), SIgA-SR (μg/min) (C) and COR (μg/dL) (D). Data in Fig. 1 were classified by subjects according to grade grouping and statistical analysis was performed. ES: 4th to 6th grade elementary school students, JHS: 7th to 9th grade junior high school students. Data were expressed as mean ± SD. NS: not significant.
induces resistance of glucocorticoid receptor function in immune cells and inhibits the regulation of HPAA against inflammation and immune response. Therefore, although SIgA decreased as a result of acute stress due to the influence of the disaster, it is considered that the action of HPAA is reduced with the passage of time and that the SIgA level gradually recovers accordingly.

In this study, significant gender differences were observed in SF and SIgA-C (Fig. 2). As for the COR and SIgA, there is no gender difference in SIgA-C in healthy subjects, and it is reported that females have significantly lower in SF and SIgA-SR than males. On the other hand, it is reported that COR is significantly lower in boys aged 8 to 18 years than girls. Viena and colleagues reported that responsiveness to acute stress by COR and SIgA varies depending on the ongoing stress state in the background. Moreover, in a previous study that examined the relationship between the state of physical activity and HPAA of children, physical activity influenced HPAA responsiveness, but the effect was gender specific. Kirschbaum et al. have also reported that the menstrual cycle affects HPAA reactivity. Furthermore, a meta-analysis of children suffering from large-scale disasters reported that girls showed more PTSD symptoms than boys. Therefore, girls may be more sensitive to chronic stress than boys in such disasters. According to the results of the present study, there was no gender difference in COR, but our previous study reported that there was a gender difference in the physical activity level of disaster-afflicted children and adolescents, and the difference continued after the disaster. Therefore, it is considered that the difference between physical activity level and chronic stress sensitivity caused a difference in SF, and a concomitant change in SIgA-C.

There was a marked difference between elementary and junior high school students in the variation of COR as a chronic stress marker (Fig. 3D). Iwadare et al. reported symptoms of PTSD and depression among elementary and junior high school students in the affected areas due to the effects of the disaster. In addition, these symptoms gradually decreased with time after the disaster. However, the status of recovery of these symptoms was different between elementary and junior high school students. Moreover, in the report on psychosomatic health of elementary and junior high school students after the 1995 Great Hanshin-Awaji earthquake, although emotions such as fear and anxiety appeared early after the disaster, recovery was also observed relatively early. However, depressive and psychophysical symptoms were slower to appear and continued for a longer time, and this tendency was more marked in junior high school students and female students. The physical activity level of elementary and junior high school students in the area affected by the 2011 Great East Japan earthquake and tsunami also declined over several years in the aftermath of the disaster, and this tendency was also more marked in junior high school students and female students. Accordingly, it is hard to say whether the life environment of the disaster area has fully recovered, even after 5 years; and it is speculated that junior high school students, who had mentally unstable periods of puberty during this time, had a greater accumulation of mental influence than elementary school students. As a result, since a chronic increase in COR will be a risk factor for developing PTSD in the future, observation of COR, some kind of system for checking for symptoms of PTSD and coping mechanisms for stress (e.g. positive coping, social support) may need to be developed as a countermeasure in the future.

In addition, the age-dependent increase in SIgA concentration has been studied extensively. It has been found that SIgA increases significantly from infancy to school age, but no significant difference has been observed in age-related changes in elementary and junior high school students.

Regarding the relationship between COR and SIgA, COR and SIgA are generally recognized as stress and immune biomarkers, respectively. Similarly, there are also many reports of COR and SIgA independently functioning as stress biomarkers. In addition, we anticipated SIgA as a counterpart of COR fluctuations in this study. Indeed, the fluctuation of COR and SIgA at the time of waking and diurnal variation show corresponding variations. It has been reported in the SIgA regulatory mechanism of COR that COR downregulates the expression of the poly-Ig receptor, as an IgA transporter, on mucosal surfaces in a short period of time, and suppresses the activity of IgA-secreting B cells over several days, thus COR has acute and chronic effects on both IgA-secreting and the oral secretion machinery. In addition, it has been shown that other neuroendocrine factors such as catecholamines and neurotransmitters also participate in the regulation of SIgA at the same time. On the other hand, there have been few reports of long-term fluctuations for elementary and junior high school students who suffered from natural disasters like this time. Because our results showed no correlation between COR and SIgA among the affected elementary and junior high school students, it is suggested that the long-term chronic stress condition caused by the disaster may have caused some change in the interaction between the neuroendocrine system and the immune system.

We should mention several limitations of this study. First, because the design was a continuous cross-sectional study, it is not possible to refer to the consequences of the results. Also, because this study was conducted in only one target area, it is necessary to judge carefully whether the results would be similar to other areas. Moreover, because there are no baseline data from before the disaster, changes in the measured items cannot be assessed. We think that establishing an appropriate control group would be necessary to examine the effects of the earthquake and tsunami disaster. In this study, we evaluated the effect.
of chronic stress, but need to consider the effect of acute stress as well.

In conclusion, the salivary chronic stress and immune biomarkers in elementary and junior high school students changed during the 4-year period after the disaster. Notably, the fluctuation of cortisol as a chronic stress biomarker and the rise of S IgA as an immune biomarker were observed; but the observed changes of the two biomarkers did not correspond. Thus, factors influencing the indicators, such as gender differences, school grade level, physical activity, and quality of life, may differ. In the future, we will examine the details of factors that affect chronic stress and immune biomarkers, and also consider simultaneously whether it is possible to relieve excessive chronic stress situations such as this one in some way. It is important to identify the measures for reducing chronic stress in children as early as possible to protect the health of children in the affected areas. Although sufficient verification is still necessary, monitoring of cortisol may possibly lead to opportunities and measures to prevent PTSD development. Such are important for healthy growth of mind and body of children, maintenance and promotion of health, and further acquisition of future quality of life.

Conflict of Interests

The authors declare that they have no conflict of interests.

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References

1) Mimura N, Yasuhara K, Kawagoe S, Yokoki H and Kazama S. 2011. Damage from the Great East Japan Earthquake and Tsunami - A quick report. Mitig Adapt Strat Glob Change 16: 803-818. doi: 10.1007/s11027-011-9297-7.
2) Kotozaki Y and Kawashima R. 2012. Effects of the Hi-gashi-Nihon Earthquake: posttraumatic stress, psychological changes, and cortisol levels of survivors. PLoS One 7: e34612. doi: 10.1371/journal.pone.0034612.
3) Yabe H, Suzuki Y, Mashiko H, Nakayama Y, Hisata M, Niwa S, Yasumura S, Yamashita S, Kamiya K and Abe M. 2012. Mental Health Group of the Fukushima Health Management Survey. 2014. Psychological distress after the Great East Japan Earthquake: The Fukushima Health Management Survey. Nihon Kosha Eisei Zasshi 63: 3-10. doi: 10.11236/jph.63.1 3.
4) Ohira T, Hosoya M, Yasumura S, Satoh H, Suzuki H, Sakai A, Ohtsuru A, Kawasaki Y, Takahashi A, Ozasa K, Kobashi G, Kamiya K, Yamashita S and Abe M. 2016. Effect of evacuation on body weight after the Great East Japan Earthquake. Am J Prev Med 50: 553-560. doi: 10.1016/j.amepre.2015.10.008.
5) Nagai M, Ohira T, Yasumura S, Takahashi H, Yuki M, Nakano H, Wen Z, Yabe H, Ohtsuru A, Maeda M and Takase K. 2016. Association between evacuation condition and habitual physical activity in Great East Japan Earthquake evacuees: The Fukushima Health Management Survey. Prog Brain Res 220: 233-246. doi: 10.1016/bs.prbr.2016.05.002.
6) Pervanidou P and Chrousos GP. 2012. Posttraumatic stress disorder in children and adolescents: neuroendocrine perspectives. Sci Signal 5: p6. doi: 10.1136/bmj.2003327.
7) Lupien SJ, de Leon M, de Santi S, Convit A, Tarshish C, Nair NP, Thakur M, McEwen BS, Hauger RL and Meaney MJ. 1998. Cortisol levels during human aging predict hippocampal atrophy and memory deficits. Nat Neurosci 1: 69-73. doi: 10.1038/271.
8) McAuley MT, Kenny RA, Kirkwood TB, Wilkinson DJ, Jones JJ and Miller VM. 2009. A mathematical model of aging-related and cortisol induced hippocampal dysfunction. BMC Neurosci 10: 26. doi: 10.1186/1471-2202-10-26.
9) Woon FL, Sood S and Hedges DW. 2010. Hippocampal volume deficits associated with exposure to psychological trauma and posttraumatic stress disorder in adults: a meta-analysis. Prog Neuropsychopharmacol Biol Psychiatry 34: 1181-1188. doi: 10.1016/j.pnpbp.2010.06.016.
10) Song Y, Zhou D and Wang X. 2008. Increased serum cortisol and growth hormone levels in earthquake survivors with PTSD or subclinical PTSD. Psychoneuroendocrinology 33: 1155-1159. doi: 10.1016/j.psyneuen.2008.05.005.
11) Pervanidou P, Kolaitis G, Charitaki S, Margeli A, Ferentinou S, Bakoula C, Lazaropoulou C, Papassotiriou I, Tsiantis J and Chrousos GP. 2007. Elevated morning serum interleukin (IL)-6 or evening salivary cortisol concentrations predict posttraumatic stress disorder in children and adolescents six months after a motor vehicle accident. Psychoneuroendocrinology 32: 991-999. doi: 10.1016/j.psyneuen.2007.07.001.
12) Granditsaeg P and Johansen FE. 2005. Mucosal B cells: phenotypic characteristics, transcriptional regulation, and homing properties. Immunol Rev 206: 32-63. doi: 10.1111/j.0105-2896.2005.00283.x.
13) Strugnell RA and Wijburg OL. 2010. The role of secretory antibodies in infection immunity. Nat Rev Microbiol 8: 656-667. doi: 10.1038/nrmicro2384.
14) Mackinnon LT, Ginn E and Seymour GJ. 1993. Decreased salivary immunoglobulin A secretion rate after intense interval exercise in elite kayakers. Eur J Appl Physiol Occup Physiol 67: 180-184.
15) Cunningham-Rundles C and Bodian C. 1999. Common variable immunodeficiency: clinical and immunological features of 248 patients. Clin Immunol 92: 34-48. doi: 10.1006/ clin.1999.4725.
16) Mackinnon LT and Jenkins DG. 1993. Decreased salivary immunoglobulins after intense interval exercise before and after training. Med Sci Sports Exerc 25: 678-683.
17) Dimitriou L, Sharp NC and Doherty M. 2002. Circadian effects on the acute responses of salivary cortisol and IgA in well trained swimmers. Br J Sports Med 36: 260-264. doi: 10.1136/bjsm.36.4.260.
18) Cohen S, Miller GE and Rabin BS. 2001. Psychological stress and antibody response to immunization: a critical re-
view of the human literature. Psychosom Med 63: 7-18.
19) Murphy L, Denis R, Ward CP and Tartar JL. 2010. Academic stress differentially influences perceived stress, salivary cortisol, and immunoglobulin-A in undergraduate students. Stress 13: 365-370. doi: 10.1039/i01053891003615473.
20) Usami M, Iwadare Y, Watanabe K, Kodaira M, Ushijima H, Tanaka T, Harada M, Tanaka H, Sasaki Y and Saito K. 2014. Decrease in the traumatic symptoms observed in child survivors within three years of the 2011 Japan earthquake and tsunami. PLoS One 9: e110898. doi: 10.1371/journal.pone.0110898.
21) Iwadare Y, Usami M, Suzuki Y, Ushijima H, Tanaka T, Watanabe K, Kodaira M and Saito K. 2014. Posttraumatic symptoms in elementary and junior high school children after the 2011 Japan earthquake and tsunami: symptom severity and recovery vary by age and sex. J Pediatr 164: 917-921.e1. doi: 10.1016/j.jpeds.2013.11.061.
22) Laurent HK, Stroud LR, Brush B, D’Angelo C and Granger PA. 2015. Secretory IgA reactivity to social threat in youth: Relations with HPA, ANS, and behavior. Psychoneuroendocrinology 59: 81-90. doi: 10.1016/j.psyneuen.2015.04.021.
23) Wright BJ, O’Brien S, Hazi A and Kent S. 2014. Increased systolic blood pressure reactivity to acute stress is related with better self-reported health. Sci Rep 4: 6882. doi: 10.1038/srep06882.
24) Sakamoto Y, Ueki S, Shimakami H, Kasai T, Takato J, Ozaki H, Kawakami Y and Haga H. 2005. Effects of low-intensity physical exercise on acute changes in resting saliva secretory IgA levels in the elderly. Geriatr Gerontol Int 5: 202-206. doi: 10.1111/j.1447-0594.2005.00297.x.
25) Usami M, Iwadare Y, Watanabe K, Kodaira M, Ushijima H, Tanaka T, Harada M, Tanaka H, Sasaki Y and Saito K. 2014. Analysis of changes in traumatic symptoms and daily life activity of children affected by the 2011 Japan earthquake and tsunami over time. PLoS One 9: e88885. doi: 10.1371/journal.pone.0088885.
26) Phillips AC, Carroll D, Evans P, Bosch JA, Clow A, Hucklebridge F and Der G. 2006. Stressful life events are associated with low secretion rates of immunoglobulin A in saliva in the middle aged and elderly. Brain Behav Immun 20: 191-197. doi: 10.1016/j.bbi.2005.06.006.
27) Stratakis CA and Chrousos GP. 1995. Neuroendocrinology and pathophysiology of the stress system. Ann N Y Acad Sci 771: 1-18.
28) van Rood Y, Bogarda M, Goulmy E and Houwelingen H. 1993. The effects of stress and relaxation in vitro immune response in man: a meta-analytic study. J Behav Med 16: 163-181.
29) Cohen S, Janicki-Deverts D, Doyle WJ, Miller GE, Frank E, Rabin BS and Turner RB. 2012. Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk. Proc Natl Acad Sci USA 109: 5995-5999. doi: 10.1073/pnas.11118355109.
30) Evans P, Der G, Ford G, Hucklebridge F, Hunt K and Lambert S. 2000. Social class, sex, and age differences in mucosal immunity in a large community sample. Brain Behav Immun 14: 41-48. doi: 10.1006/brbi.1999.0571.
31) Watanabe Y, Mizoguchi H, Masamura K and Nagaya T. 1997. No relationship of salivary flow rate or secretory immunoglobulin A to dental caries in children. Environ Health Prev Med 2: 122-125. doi: 10.1007/BF02931977.
32) van der Voorn B, Hollander JJ, Ket JCF, Rotteveel J and Finken MJ. 2017. Gender-specific differences in hypothalamic-pituitary-adrenal axis activity during childhood: a systematic review and meta-analysis. Biol Sex Differ 8: 3. doi: 10.1186/s13293-016-0123-5.
33) Viena TD, Banks JB, Barbu IM, Schulman AH and Tartar JL. 2012. Differential effects of mild chronic stress on cortisol and S-IgA responses to an acute stressor. Biol Psychol 91: 307-311. doi: 10.1016/j.biopsycho.2012.08.003.
34) Martikainen S, Posenen AK, Lahti J, Heinonen K, Pyhala R, Tammelin T, Kajantie E, Strandberg TE, Reynolds RM and Raikkonen K. 2014. Physical activity and hypothalamic-pituitary-adrenocortical axis function in adolescents. Psychoneuroendocrinology 49: 96-105. doi: 10.1016/j.psyneuen.2014.06.023.
35) Kirschbaum C, Kudielka BM, Gaab J, Schommer NC and Hellhammer DH. 1999. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamic-pituitary-adrenal axis. Psychosom Med 61: 154-162.
36) Trickey D, Siddaway AP, Meiser-Stedman R, Serpell L and Field AP. 2012. A meta-analysis of risk factors for post-traumatic stress disorder in children and adolescents. Clin Psychol Rev 32: 122-138. doi: 10.1016/j.cpr.2011.12.001.
37) Okazaki K, Suzuki K, Sakamoto Y and Sasaki K. 2015. Physical activity and sedentary behavior among children and adolescents living in an area affected by the 2011 Great East Japan earthquake and tsunami for 3 years. Prev Med Rep 2: 720-724. doi: 10.1016/j.pmedr.2015.08.010.
38) Shioyama A, Uemoto M, Shinfuku N, Ide H, Seki W, Mori S, Inoue S, Natsuno R, Asakawa K and Osabe H. 2000. The mental health of school children after the Great Hanshin-Awaji Earthquake: II. Longitudinal analysis. Seishin Shinkeigaku Zassi 102: 481-497 (in Japanese).
39) Andrews G, Tennant C, Hewson DM and Vaillant GE. 1978. Life event stress, social support, coping style, and risk of psychological impairment. J Nerv Ment Dis 166: 307-316.
40) Du B, Ma X, Ou X, Jin Y, Ren P and Li J. 2018. The prevalence of posttraumatic stress in adolescents eight years after the Wenchuan earthquake. Psychiatry Res 262: 262-269. doi: 10.1016/j.psychres.2018.02.019.
41) Altindag A, Ozen S and Sir A. 2005. One-year follow-up study of posttraumatic stress disorder among earthquake survivors in Turkey. Compr Psychiatry 46: 328-333. doi: 10.1016/j.comppsych.2005.01.005.
42) Jafarzadeh A, Sadeghi M, Karam GA and Vazirinejad R. 2010. Salivary IgA and IgE levels in healthy subjects: relation to age and gender. Braz Oral Res 24: 21-27.
43) Sonesson M, Hamberg K, Wallengren ML, Masson L and Ericson D. 2011. Salivary IgA in minor-gland saliva of children, adolescents, and young adults. Eur J Oral Sci 119: 15-20. doi: 10.1111/j.1600-0722.2010.00794.x.
44) Hucklebridge F, Clow A and Evans P. 1998. The relationship between salivary secretory immunoglobulin A and cortisol: neuroendocrine response to awakening and the diurnal cycle. Int J Psychophysiol 31: 69-76. doi: 10.1016/S0167-8760(98)00042-7.
45) Miletic ID, Schiffman SS, Miletic VD and Sattely-Miller EA. 1996. Salivary IgA secretion rate in young and elderly persons. Physiol Behav 60: 243-248. doi: 10.1016/0031-9384(95)02161-2.
46) Jarillo-Luna A, Rivera-Aguilar V, Garfias HR, Lara-Padilla
E, Kormanovsky A and Campos-Rodriguez R. 2007. Effect of repeated restraint stress on the levels of intestinal IgA in mice. Psychoneuroendocrinology 32: 681-692. doi: 10.1016/j.psyneuen.2007.04.009.

47) Rosato R, Jammes H, Belair L, Puissant C, Kraehenbuhl JP and Djiane J. 1995. Polymeric-Ig receptor gene expression in rabbit mammary gland during pregnancy and lactation: evolution and hormonal regulation. Mol Cell Endocrinol 110: 81-87.

48) Martinez-Carrillo BE, Godinez-Victoria M, Jarillo-Luna A, Oros-Pantoja R, Abarca-Rojano E, Rivera-Aguilar V, Yepez JP, Sanchez-Torres LE and Campos-Rodriguez R. 2011. Repeated restraint stress reduces the number of IgA-producing cells in Peyer’s patches. Neuroimmunomodulation 18: 131-141. doi: 10.1159/000322625.

49) Teeuw W, Bosch JA, Veerman EC and Amerongen AV. 2004. Neuroendocrine regulation of salivary IgA synthesis and secretion: implications for oral health. Biol Chem 385: 1137-1146. doi: 10.1515/BC.2004.147.