SALIVA CORTISOL AND DEHYDROEPIANDROSTERONE (DHEA) AS BIOCHEMICAL INDICATOR TO EVALUATE JOB STRESS

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A THEMATIC PAPER SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PUBLIC HEALTH FACULTY OF GRADUATE STUDIES MAHIDOL UNIVERSITY 2013

Thematic Paper
entitled

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was submitted to the Faculty Graduate Studies, Mahidol University
for the degree of Master of Public Health
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ACKNOWLEDGEMENTS

I sincerely appreciate Assoc. Prof. Dr. Nopporn Howteerakul, my advisor, and Assoc. Prof. Dr. Nawarat Suwannapong, my co-advisor, for spending their time and energy instructing me and reviewing my thematic paper. I also would like to express my best gratitude to Dr. Thitipat Rajatanun for providing useful comments and suggestions at my thesis proposal and final defense.

Here I would like to mention Prof. Yuichiro Ono of Fujita Health University, Japan, to whom I used to report. He has dedicated himself for more than 30 years to research with regard to prevention of musculoskeletal disorders among workers. His research works inspired me to make a start of the present study. He kindly wrote a recommendation so that I became a student of Master of Public Health course.

I would like to thank all teachers and staff of the Faculty of Public Health, Mahidol University for their kind and heartwarming support. All my classmates and friends in Thailand helped me get over the difficulties in my study. We shared our experience to each other.

Finally, I thank my family: Sachiko, Momoka, Masahiko, Shigeru, and Hatsu. Their emotional support is an indispensable piece to complete my thesis work.

The present research is granted by the Promotion and Mutual Aid Corporation for Private Schools of Japan and the Japan Society for the Promotion of Science.

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ABSTRACT

Job stress is known as a risk factor of health disorders. The purpose of this study is to examine whether saliva cortisol, dehydroepiandrosterone (DHEA), and cortisol/DHEA ratio (C/D ratio) are the biochemical indicators to evaluate job stress. The subjects were 115 female nursery school teachers. Job stress was evaluated using the Effort-Reward Imbalance model: effort (high or low), reward (high or low), effort-reward imbalance (high or low), overcommitment to work (high or low), and combination of effort-reward imbalance and overcommitment (high, intermediate, or low). Cortisol and DHEA in saliva collected at 9:00hr, 12:00hr, and 15:00hr in a working day were measured with the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Repeated-measured analysis of variance revealed that all the job stress indicators showed insignificant interaction by time ($p > 0.05$) and main effect ($p > 0.05$) on saliva cortisol, DHEA, or C/D ratio. The present study does not exhibit significant associations between job stress and saliva cortisol, DHEA, and C/D ratio. Daytime saliva cortisol, DHEA, and C/D ratio may not work as the biochemical indicators to evaluate job stress.

KEY WORDS: JOB STRESS / EFFORT-REWARD IMBALANCE MODEL / SALIVA / CORTISOL / DEHYDROEPIANDROSTERONE (DHEA)

59 pages

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

ACR	Awakening cortisol response
ACTH	Adrenocorticotropic hormone
C/D ratio	Cortisol/DHEA ratio
CRH	Corticotropin-releasing hormone
DHEA	Dehydroepiandrosterone
ERI	Effort-Reward Imbalance
E/R ratio	Effort/Reward ratio
HPA	Hypothalamic-pituitary-adrenocortical
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
OC	Overcommitment to work
SD	Standard deviation

CHAPTER I
INTRODUCTION

1.1Background and justification

Stress is an important public health issue at present. The National Survey on Lifestyle Preferences revealed that nearly 60% of Japanese people felt stress very much or to some extent[1]. Job stress, which is the stress caused by social, behavioral, environmental, and cognitive stressors at work[2], induces severe health disorders such as coronary heart diseases[3], mental disorders[4], and musculoskeletal disorders[5, 6]. From a public health viewpoint, screening stressed-over people is expected as an effective way to prevent such stress-induced health disorders. Reviews showed the effectiveness of stress management programs for employees on reduction in their complaint and clinical visits due to psychiatric disorders[7, 8].

There are two ways to evaluate job stress: subjective and objective methods. There are some subjective methods whose reliability and validity have been established. For example, the Effort-Reward Imbalance (ERI) model[2] is one of the representative subjective methods. This model focuses on working conditions as the source of social, behavioral, environmental, and cognitive stressors of job stress. Job stress is evaluated through the concepts of effort, reward, effort-reward imbalance, and overcommitment.

On the other hand, objective methods to evaluate stress are still on the way of progress. Saliva cortisol and dehydroepiandrosterone (DHEA) are expected as the promising indicator. Acute stress stimulates the hypothalamic-pituitary-adrenocortical (HPA) axis, causing hyper-secretion of cortisol[9]. DHEA worked as an antagonist

against cortisol[10]. More concise measurement of cortisol and DHEA in saliva is available by introducing the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method [11-13]. However, it is still unknown whether saliva cortisol and DHEA can be the appropriate biochemical indicator to evaluate job stress.

It is reported that female nursery school teachers are so stressed over that they frequently suffer from musculoskeletal disorders, a representative consequence of exposure to stress[14]. Therefore, they were chosen as the subjects of the present study. They are exposed not only to physical stress but to social (e.g., interaction between teachers and children and that between teachers and children's parents), behavioral, environmental, and cognitive stress.

1.2Objectives

1. To examine whether saliva cortisol and DHEA are the biochemical indicators to evaluate job stress,

1.3Research hypotheses

1. Exposure to job stress increases saliva cortisol concentration at daytime among female nursery school teachers;
2. Exposure to job stress decreases saliva DHEA concentration at daytime among female nursery school teachers;
3. Exposure to job stress increases saliva cortisol/DHEA ratio (C/D ratio) at daytime among female nursery school teachers.

1.4Variables

Independent variables:

- Job stress
  - Effort score
  - Reward score
  - Effort/Reward ratio (E/R ratio)
  - Overcommitment to work score (OC score)
  - Combination of E/R ratio and OC score
- Measurement time
  - 9:00hr
  - 12:00hr
  - 15:00hr

Dependent variables:

- Saliva cortisol
- Saliva DHEA
- Saliva C/D ratio

1.5Operational definitions

Job stress refers to stress caused by social, behavioral, environmental, and cognitive stressors of the nursery school teachers. The ERI model[(2)] was employed for evaluation of job stress.

Effort score is a job stress indicator calculated by applying the Japanese version of the ERI Questionnaire[(15)] to the nursery school teachers. Effort is expended as a part of a socially organized exchange process to which society at large contributes in terms of occupational rewards. Those with a high effort score are regarded to be

stressful.

Reward score is a job stress indicator calculated by applying the Japanese version of the ERI Questionnaire[15] to the nursery school teachers. Reward is distributed in the form of money, esteem, opportunities, and/or job security. Those with a low reward score are regarded to be stressful.

E/R ratio is a job stress indicator calculated by dividing an effort score by a reward score for each nursery school teacher. Those with a high E/R ratio are regarded to be stressful.

OC score is a job stress indicator calculated by applying the Japanese version of the ERI Questionnaire[15] to the nursery school teachers. Overcommitment to work is defined as a set of attitudes, behaviors, and emotions that reflect excessive endeavor combined with a strong desire for approval and esteem. Those with a high OC score are regarded to be stressful.

Combination of E/R ratio and OC score is a job stress indicator defined by an E/R ratio and an OC score for each nursery school teacher. Those at a high level of combination of E/R ratio and OC score are regarded to be stressful.

Saliva cortisol is cortisol existing in saliva collected from the nursery school teachers. Saliva cortisol concentration is measured with LC-MS/MS method. The measurement was performed by ASKA Pharma Medical Co. Ltd., Kawasaki, Japan. High saliva cortisol concentration is supposed to reflect a high level of the HPA activity.

Saliva DHEA is DHEA existing in saliva collected from the nursery school teachers. Saliva DHEA concentration is measured with LC-MS/MS method. The measurement was performed by ASKA Pharma Medical Co. Ltd., Kawasaki, Japan.

Saliva C/D ratio is the ratio of concentration of saliva cortisol to that of

saliva DHEA. As well as saliva cortisol, high saliva C/D ratio is supposed to reflect a high level of the HPA activity.

LC-MS/MS method is a method adopted to measure the concentration of saliva cortisol and DHEA of the nursery school teachers. The method used the combined devices of liquid chromatography and tandem-connected mass spectrometry.

Nursery schools are facilities to care children aged between 0 and 6 while their parents are working.

Nursery school teachers were employees who take care of children aged between 0 and 6 in the nursery school.

Employment status:

- **Regular staffs** work full-time without a contract regarding the period of employment.
- **Temporary workers** work full-time or part-time with a contract of a certain period of employment, usually a one-year contract. Salaries of them were generally lower than those of the regular staffs. There was little difference in the job between the regular staffs and temporary workers.

Current smoking was defined as smoking at least one cigarette a day.

Ovulatory phase was defined as the period during which an egg cell (ovum) is released from the ovary.

Menstruation irregularity was defined as the status that menstruation does not start regularly.

Health problems are the obstructions to normal function of the nursery school teachers. For the present study, it was identified whether they had musculoskeletal disorders, dental and gum diseases, and other health problems.

Musculoskeletal disorders included upper extremity (arm, shoulder, and

hand) and back symptoms. For each part, the nursery school teachers were classified into three levels based on the subjective symptoms: those with no, moderate, and severe symptoms.

Dental and gum diseases were defined as diseases which the nursery schools teachers visited a dentist to treat.

Other health problems were health problems other than musculoskeletal and dental and gum diseases.

Medicine that could affect secretion of cortisol and/or DHEA included estrogen (typically used for treatment of infertility), oral contraceptive pill, steroid (typically used for atopic dermatitis and asthma), and anti-depression medicine.

Blood contamination in saliva was judged with the measured value of saliva cortisol and DHEA. Concentrations of both saliva cortisol and DHEA are about 30-50 times higher than those of serum cortisol and DHEA[10, 16]. When blood contamination occurs, both of measured values of cortisol and DHEA rise up. Considering these facts, the author set the inter- and intra-personal standards to judge blood contamination in saliva as follows:

- Inter-personal standard: If both of the measured values of saliva cortisol and DHEA were higher than the means + 2 SDs, blood contamination in saliva was suspected.
- Intra-personal standards: If both of the measured values of saliva cortisol and DHEA at one measurement time were 30 or more times higher than those at other measurement time, blood contamination in saliva was suspected.

1.6Conceptual framework

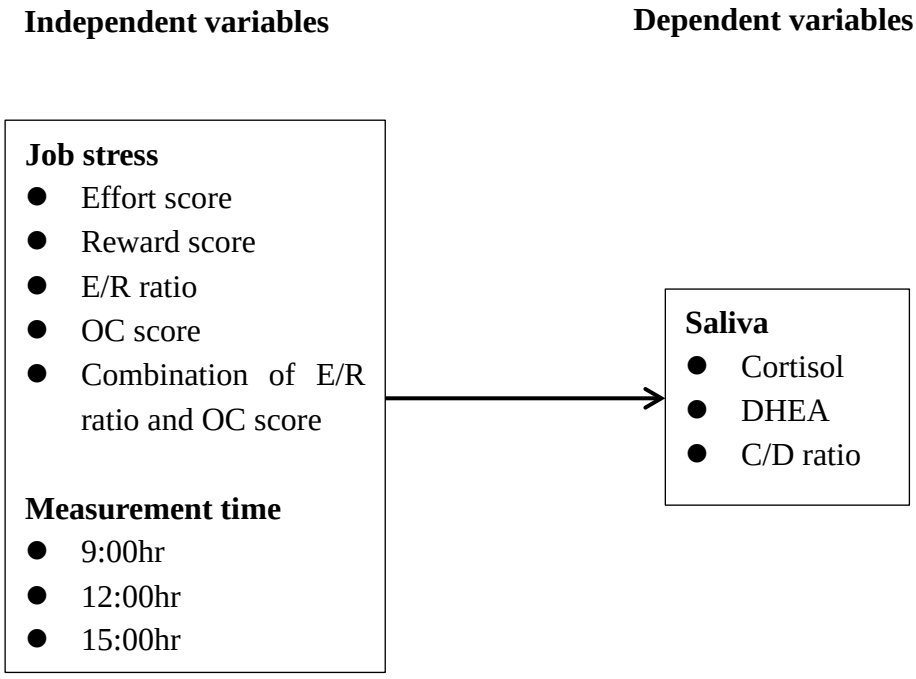

Figure 1.1 Conceptual framework: saliva cortisol and DHEA as biochemical indicator to evaluate job stress

CHAPTER II
LITERATURE REVIEW

Since the study object is to examine whether saliva cortisol and DHEA are the biochemical indicators to evaluate job stress, the literature review covered saliva cortisol, DHEA, and C/D ratio, job stress, and the ERI model.

2.1 Saliva Cortisol and DHEA

Cortisol[9] is a glucocorticoid and produced at adrenal gland. It is released into blood flow in response to stress. The physiological functions are varied: increasing blood sugar; suppressing the immune system; aiding in fat, protein, and carbohydrate metabolism; and decreasing bone density. Cortisol in saliva is supposed as the surrogate of that in blood flow[16]. Cortisol is effused from blood flow into saliva at salivary gland[9, 10]. It is reported that concentration of serum cortisol is about 30-50 times higher than that of saliva cortisol[16] .

DHEA[10] is an endogenous steroid produced at adrenal glands, gonads, and brain. DHEA works as a metabolic intermediate in the biosynthesis of the androgen and estrogen sex steroids. Recent studies indicate that DHEA also has a variety of potential biological effects including balancing to cortisol. DHEA in saliva is also supposed to reflect the amount of those in blood flow[16]. DHEA is effused from blood flow into saliva at salivary gland[9, 10].

2.2 Cortisol, DHEA, and their relationship to stress

Acute stress stimulates the HPA axis, leading to secretion of cortisol[9]. When a human being is exposed to acute stress, hypothalamus releases corticotropin-releasing hormone (CRH). CRH stimulates anterior pituitary to secret adrenocorticotropic hormone (ACTH). ACTH makes adrenal grand secretion cortisol.

DHEA works as an antagonist against cortisol and a suppressor of cortisol stress response[10]. Therefore, some researchers insist that C/D ratio would be a better indicator than a single use of cortisol in evaluating the degree of HPA activity[17, 18].

2.3 Application of LC-MS/MS method for quantification of cortisol and DHEA

Immunoassay often fails to differentiate cortisol from chemicals that are structurally similar to cortisol, such as cortisone and prednisolone, possibly causing incorrect quantification of cortisol. LC-MS/MS method identifies cortisol and DHEA for the quantification more correctly than immunoassay[11-13].

2.4 Job stress

Unlike the era when Selye advocated his stress theory[19], it is generally regarded that stress includes not only biological but psychological contexts. Job stress is supposed a kind of psychosocial stress caused by social (e.g., person-person, person-family, and person-peer group interaction), behavioral, environmental, and cognitive stressors at work[2].

2.5 The ERI model and ERI questionnaire

The concept of the ERI model is drawn at Figure 2.1. The ERI model[2] focuses on both situational and personal conditions at work. This model defines stressful experience as an imbalance between effort and reward. Effort is expended as a part of a socially organized exchange process to which society at large contributes in terms of occupational rewards. Reward is distributed in the form of money, esteem, opportunities, and/or job security. Effort-reward imbalance is defined as the status in which employees make much effort while they are insufficiently rewarded. Overcommitment to work is defined as a set of attitudes, behaviors, and emotions that reflect excessive endeavor combined with a strong desire for approval and esteem. Those who overcommit to work exaggerate their efforts beyond levels that are usually supposed appropriate. It is hypothesized that overcommitment to work induces emotional exhaustion and amplifies adverse effects of effort-reward imbalance.

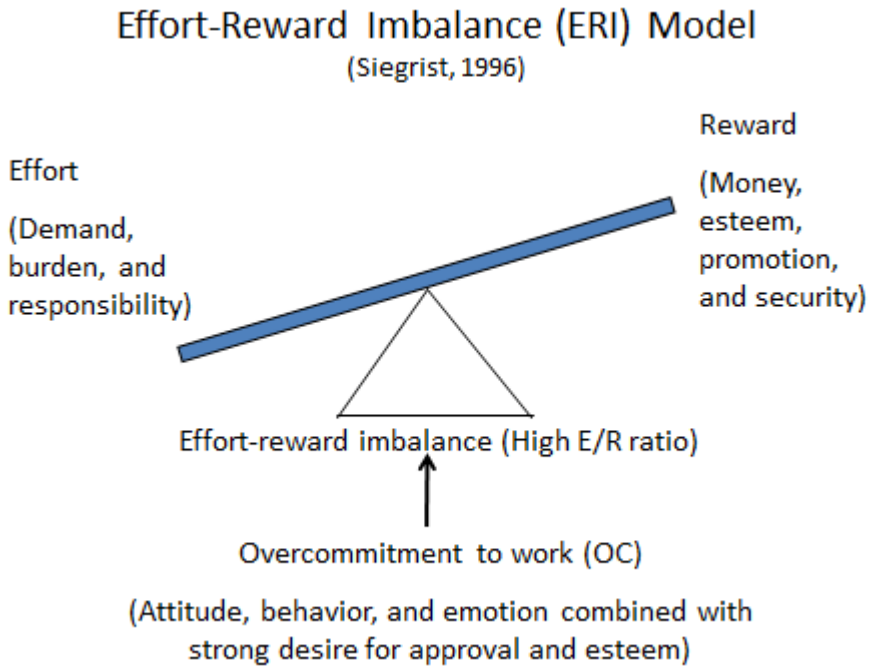

Figure 2.1 Concept of the ERI model

Reviews proved that effort-reward imbalance and overcommitment to work are related to coronary heart diseases[3], mental disorders[4], and musculoskeletal disorders[5, 6].

The ERI questionnaire was developed in order to evaluate job stress with the ERI model. The ERI questionnaire was translated into Japanese by Tsutsumi et al.[15] The Japanese version of the ERI Questionnaire consists three parts to evaluate effort, reward, and overcommitment to work (Table 2.1; see also Appendices A and B). The reliability and validity were tested. Tsutsumi et al.[15] tested the validity during the developing process. Ota et al.[20] reported that the Cronbach's alpha coefficients of effort, reward, and overcommitment were 0.83, 0.86, and 0.76, respectively.

2.6 Effects of job stress evaluated with the ERI model on saliva cortisol, DHEA, and C/D ratio

Researchers have tried to prove that job stress evaluated with the ERI model lead to increase in saliva cortisol. However, the previous findings were not concordant.

Eller et al.[21] found that effort-reward imbalance increases cortisol secretion. In contrary, Maina et al.[22] reported that effort-reward imbalance decreases cortisol secretion. Some researchers indicated insignificant association between E/R ratio and cortisol secretion[23-25].

Eller et al.[21] and Steptoe et al.[24] exhibited that overcommitment to work did not increase saliva cortisol level in female but in male. Maina et al.[22] reported that overcommitment to work did not increase saliva cortisol level both in male and female.

Previous studies did not find significant effects of working environment, such as job demand, job control, social support at work, and organizational changes,

Table 2.1 The Japanese version of the ERI Questionnaire

Effort scales:
eq1. I have constant time pressure due to a heavy work load.
eq2. I have many interruptions and disturbances in my job.
eq3. I have a lot of responsibility in my job.
eq4. I am often pressured to work overtime.
eq5. My job is physically demanding.
eq6. Over the past years, my job has become more and more demanding.
Reward scales
rq1. I receive the respect I deserve from my superiors.
rq2. I receive the respect I deserve from my colleagues.
rq3. I experience adequate support in difficult situations.
rq4. I am treated unfairly at work.
rq5. I have experienced or I expect to experience an undesirable change in my work situation.
rq6. My job promotion prospects are poor.
rq7. My job security is poor.
rq8. My current occupational position adequately reflects my education and training.
rq9. Considering all my efforts and achievements, I receive the respect and prestige I deserve at work.
rq10. Considering all my efforts and achievements, my work prospects are adequate.
rq11. Considering all my efforts and achievements, my salary/income is adequate.
Overcommitment to work scale
oq1. I get easily overwhelmed by time pressures at work.
oq2. I start thinking about work problems as soon as I get up in the morning.
oq3. When I get home, I can easily relax and forget all about work.
oq4. People close to me say I sacrifice too much for my job.
oq5. Work is usually still on my mind when I go to bed.
oq6. If I put off something that needs to be done today, I'll have trouble sleeping at night.

on DHEA[26]. To my knowledge, the effects of job stress evaluated with the ERI model on saliva DHEA and C/D ratio are still unclear because little relevant research has adopted the ERI model.

2.7 Summary

Cortisol is supposed to reflect the HPA activity. Recent research revealed the antagonistic effects of DHEA against cortisol, suggesting that DHEA and C/D ratio are needed to be considered in evaluation of the HPA activity. Technical improvement enables precise measurement of saliva cortisol and DHEA.

Saliva cortisol, DHEA, and C/D ratio are expected as the prospective indicators of job stress under the hypothesis that stress stimulates the HPA axis. Despite the expectation, previous research presented the contradictory findings regarding the relationship between job stress evaluated with the ERI model and saliva cortisol.

CHAPTER III
MATERIALS AND METHODS

3.1Research design

A secondary data set of a prospective cohort study was cross-sectionally analyzed. The data were derived from the prospective cohort study entitled “Stress evaluated with saliva stress-related hormones and musculoskeletal disorders.” For the present study, the data set was cross-sectionally analyzed to examine the association between job stress and saliva cortisol, DHEA, and C/D ratio.

3.2 Study site, population, and sample

The subjects were recruited from 29 private nursery schools in Aichi Prefecture, Japan in the winter (from December through February) of the years between 2010 and 2012. The nursery schools had been in cooperation with the Department of Public Health, Fujita Health University School of Medicine, which the author belongs to, in terms of prevention of occupational diseases, such as musculoskeletal disorders and mental disorders.

The subjects had to meet all of the following inclusion criteria: (1) female nursery teachers, (2) aged between 20 and 49, and (3) not pregnant. Those who met one of the following exclusion criteria were excluded from analysis: (1) taking medicine that could affect secretion of cortisol and/or DHEA, such as estrogen, oral contraceptive pills, steroid, and anti-depression medicine, and (2) blood contamination in saliva.

3.3 Sample size calculation

The sample size was calculated from the following formula for comparing two means[27].

$$m = \frac{2\left(Z_{1-\alpha} + Z_{1-\beta}\right)^2}{d_t^2} + \frac{1}{2} Z_{1-\alpha}^2$$

where $\alpha = 0.05: Z_{1-\alpha} = 1.645$

$\beta = 0.05: Z_{1-\beta} = 1.282$

$d_t = \frac{|\mu_1 - \mu_2|}{\sigma}$

Saliva cortisol (nmol/l)	Those exposed to ERI	Those not exposed to ERI
Mean	18.7	17.5
Standard deviation	12.5	9.9
d_t	0.45	

The above written concentration of saliva cortisol is speculated based on a previous study which showed a significant relationship between ERI and saliva cortisol[21].

The minimum required sample size was 104, calculated as follows:

$$m = \frac{2\ (1.645 + 1.282)^2}{0.45^2} + \frac{1}{2} 1.645^2 = 104$$

3.4 Research instruments

Details of the instruments employed for the present study are described separately for the questionnaire and saliva collection.

3.4.1 Questionnaire

Information regarding general characteristics and job stress was collected through a self-reporting questionnaire (Appendices A and B). The questionnaire consisted of ten questions. General characteristics were examined with the first eight

questions (Qs1-8). Job stress was examined with the last two questions (Qs9 and 10).

3.4.1.1 Questions for evaluating general characteristics

General characteristics included age (Q1), employment status (Q2; regular staff or temporary worker), smoking status (Q3; current smoker or not), ovulatory phase (Q4), menstruation irregularity (Q4), and health problems (Qs5-8).

To assess whether the subjects were in the ovulatory phase (Q4), the length of the menstrual cycle and the first day of the last menstrual period were asked. The half-length menstrual cycle was added to first day of the last menstrual period to decide the hypothesized ovulation day. The subjects were judged as in the ovulatory phase if the data collection day was within 3 days (two-way) of the hypothesized ovulation day. This assessment was not performed for those with menstruation irregularity. Those who have an irregular menstrual cycle were defined as suffering from menstruation irregularity (Q4).

As health problems, information regarding musculoskeletal disorders, i.e., upper extremity (Q5) and back problems (Q6), and dental and gum diseases (Q7) was collected. The subjects were asked about their subjective musculoskeletal symptoms. They chose one of the following five options for each of upper extremity and back problems: (1) No; (2) Yes. But, I do not care about it at all; (3) Yes. I care about it. But, I do not need any support or treatment; (4) Yes. I need support by my family/coworkers; and (5) Yes. I need medical treatment or I am already in medical treatment. Those who chose the option no.1, nos. 2 or 3, and nos. 4 or 5 were regarded as having no, moderate, and severe subjective symptoms, respectively. Regarding dental and gum diseases, the subjects were assessed whether they took medication by a dentist at the time of examination. In addition, the subjects were also assessed whether they had any other health problems (Q8). They replied whether they took medication at the time of examination for any health problems other

than musculoskeletal disorders and dental and gum diseases.

3.4.1.2 Questions for evaluating job stress

To evaluate job stress, the Japanese version of the ERI Questionnaire[15] was adopted. Q9 is for evaluating effort and reward, while and Q10 is for evaluating overcommitment to work. The questions regarding effort and reward comprised a yes/no question followed by a four-point Likert scale. For each effort question, a sub-score of 1, 2, 3, 4, or 5 was given to an answer as follows: 1= Stressful situation does not exist; 2 = Stressful situation exist, but I am not at all distressed; 3 = Stressful situation exist, and I am somewhat distressed; 4 = Stressful situation exist, and I am distressed; and 5 = Stressful situation exist, and I am very distressed. An effort score was obtained by summing up each sub-score of the six effort questions. For each reward question, a sub-score of 1, 2, 3, 4, or 5 was given to an answer as follows: 1= Stressful situation exist, and I am very distressed; 2 = Stressful situation exist, and I am distressed; 3 = Stressful situation exist, and I am somewhat distressed; 4 = Stressful situation exist, but I am not at all distressed; and 5 = Stressful situation does not exist. A reward score was obtained by summing up each sub-score of the 11 reward questions. An E/R ratio was the ratio of an effort score to a reward score. In this process, a reward score was multiplied by a coefficient (0.5454) to adjust the unequal number of items in the effort and reward scales. The six questions regarding overcommitment to work consisted of a four-point Likert scale. For each of the questions, a sub-score of 1, 2, 3, or 4 was given to an answer as follows: 1 = strongly disagree; 2 = disagree; 3 = agree; and 4 = strongly agree. However, only the question no.3 (oq.3) adopted the reverse scoring: 1 = strongly agree; 2 = agree; 3 = disagree; and 4 = strongly disagree. An OC score is obtained by summing up each sub-score of the six questions. The formulae to calculate effort score, reward score, E/R ratio, and OC score is summarized in Table 3.1 (see also Table 2.1 The Japanese version of the

ERI Questionnaire).

Table 3.1 Formulae of effort score, reward score, E/R ratio, and OC score

Effort score = eq1 + eq2 + eq3 + eq4 + eq5 + eq6

- Calculated with 6 questions of effort scale: Possible score range: 6 – 30.
  - A sub-score of 1, 2, 3, 4, or 5 was given to each question based on the answers as follows: 1= Stressful situation does not exist; 2 = Stressful situation exist, but I am not at all distressed; 3 = Stressful situation exist, and I am somewhat distressed; 4 = Stressful situation exist, and I am distressed; and 5 = Stressful situation exist, and I am very distressed.

Reward score = rq1 + rq2 + rq3 + rq4 + rq5 + rq6+ rq7 + rq8 + rq9 + rq10 + rq11

- Calculated with 11 questions of reward scale: Possible score range: 11 – 55.
  - A sub-score of 1, 2, 3, 4, or 5 was given to each question based on the answers as follows: 1= Stressful situation exist, and I am very distressed; 2 = Stressful situation exist, and I am distressed; 3 = Stressful situation exist, and I am somewhat distressed; 4 = Stressful situation exist, but I am not at all distressed; and 5 = Stressful situation does not exist.

E/R ratio = Effort score / (Reward score $\times$ 0.5454)

OC score = oq1 + oq2 + oq3 + oq4 + oq5 + oq6

- Calculated with 6 questions of overcommitment to work scale: Possible score range: 6 – 24.
  - For the questions except oq3, a sub-score of 1, 2, 3, or 4 was given to an option as follows: 1 = strongly disagree; 2 = disagree; 3 = agree; and 4 = strongly agree. For the sub-score of oq3, the following scoring method was adopted: 1 = strongly agree; 2 = agree; 3 = disagree; and 4 = strongly disagree.

3.4.2 Saliva collection

For saliva collection, syringes to store 5 ml of saliva and a portable freezer are prepared.

3.5 Data collection

Details of the data collection for the present study are described separately for the questionnaire and saliva collection.

3.5.1 Questionnaire

The questionnaire was sent to the subjects about one week before saliva collection was performed. The subjects filled in the questionnaire by the day of saliva collection. The author or a trained assistant collected the questionnaire directly from the subject after the last saliva collection (at 15:00hr) was finished. When there were any missing responses in the questionnaire, the author or a trained assistant asked the subjects to answer to the questions.

3.5.2 Saliva collection

Saliva was collected three times, at 9:00hr, 12:00hr, and 15:00hr, in a working day. The subjects put 5 ml of saliva directly in a syringe for each collection time. The author or a trained assistant visited the nursery school of the subject and told them face to face how to put saliva into the syringe. For collecting saliva at scheduled time punctually, syringes were given to the subjects about 10 minutes before each collection time. Before providing the saliva, the subjects were asked to wash their mouth with drinking water. The subjects were also asked not to eat during 30 minutes before the saliva collection. The saliva samples were stored and frozen in a portable

freezer soon after each collection.

3.6 Data analysis

Details of the data analysis for the present study are described separately for job stress, saliva cortisol, DHEA, and C/D ratio, and statistical analysis.

3.6.1 Job stress

With regard to job stress indicators, the subjects were dichotomized at the median of the effort, reward, and OC scores and E/R ratio into two groups (high and low). The combination of E/R ratio and OC score consisted of the following three levels: high, intermediate, and low. Those with a high E/R ratio and a high OC score were regarded as at a high level of the combination of E/R ratio and OC score. Those with a low E/R ratio and a low OC score were regarded as at a low level of the combination of E/R ratio and OC score. The others were regarded as at an intermediate level of the combination of E/R ratio and OC score.

3.6.2 Saliva cortisol, DHEA, and C/D ratio

The saliva samples were sent to ASKA Pharma Medical Co. Ltd. (Kawasaki, Japan) who quantified saliva cortisol and DHEA with the LC-MS/MS method. The details of the LC-MS/MS method are as follows:

Chemicals and instruments that were used for the LC-MS/MS method were prepared as follows: cortisol and DHEA were purchased from Steraloids Inc (Newport, US). Cortisol-[9, 11, 12, 12-$^{2}H_4$] (Fcortisol-d_4) were purchased from C/D/N Isotopes Inc (Quebec, Canada). DHEA-[2, 2, 4, 6-$^{2}H_4$] (DHEA-d_4) was prepared form Aska Pharmaceutical Co., Ltd (Tokyo, Japan). Bond Elut C18 cartridge was purchased

from Varian, Inc (Walnut Creek, US). 4-dimethylaminopyridine (DMAP), 2-methyl-6-nitrobenzoic anhydride (MNBA), and picolinic acid (PA) were bought from Tokyo Chemical Industry (Tokyo, Japan). Triethylamine (TEA) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Cadenza CD-C18 column (250 mm x 3 mm I.D., 3μm) was purchased from Imtakt Corporation (Kyoto, Japan).

After being thawed, the collected saliva samples were centrifuged at 4 °C and 3,000 rpm for 10 min. The obtained supernatant was used for analysis.

For the measurement of cortisol and DHEA in saliva, an API-4000 triple stage quadrupole mass spectrometer (Applied Biosystems; Foster City, CA) connected to Agilent 1100 (Agilent Technologies; Santa Clara, CA), HTC-PAL (CTC Analytics AG; Zwingen, Switzerland), and ESI ion source device was employed. Column was a Cadenza CD-C18 column and used at 40 °C. The mobile phase consisted of 0.1 % formic acid (Solvent A) and acetonitrile (Solvent B). For gradient elution, A/B was used as 50/50 to 40/60 between 0 and 1.0 min, 40/60 to 10/90 between 1.0 and 4.0 min, and 10/90 to 0/100 between 4.0 and 9.0 min, 0/100 between 9.0 and 12 min, 0/100 to 50/50 between 12.00 and 12.01, and 50/50 between 12.01 and 15 min. The flow rate was 0.4 ml/min. The following ESI conditions were used: spray voltage: 4,500 V, nitrogen collision gas: 45 psi, curtain gas: 11 psi, ion source temperature: 450 °C, and ion polarity: positive.

The derivatization reagents were prepared as follows: Ten milligrams of DMAP, 20 mg of MNBA, and 25 mg of PA were dissolved into 1 ml of tetrahydrofuran, and then agitated. After 10 min, the reagent solution was used.

To human saliva (0.5 to 1 ml), DHEA-d_4 (1 ng) and cortisol-d_4 (1 ng) were added as an internal standard (IS) and with purified water to make up a volume of 1 ml. Ethyl acetate (4 ml) was added to the saliva sample, and the mixture was shaken for 10 min. Then, the organic layer was separated after frozen of aqueous phase, and the

separated organic layer was then evaporated to dryness at 45 °C with a centrifugation evaporator. The residue obtained was dissolved in the reagent solution prepared as described above (100 μl). To this mixture was added TEA (20 μl), and then the resulting mixture was allowed to stand at room temperature for 30 min. After dilution of the reaction mixture with 1 % acetic acid solution (v/v, 1 ml) to stop the reaction, the resulting mixture was loaded onto Bond Elut C18 cartridge, which had been pre-conditioned with methanol (6 ml) and purified water (6 ml). The cartridge was washed with purified water (1 ml), and then with 30 % acetonitrile solution (v/v, 2 ml). Subsequently, the derivatives were eluted with 80 % acetonitrile solution (v/v, 3 ml). After the solvent was evaporated to dryness using a centrifugation evaporator at 53-55 °C, the residue was dissolved in 40 % acetonitrile solution (v/v, 100 μl), and 20 μl-aliquot of the solution was subjected to LC-MS/MS. For quantification of cortisol and DHEA, ion transitions of *m/z* 468.2/309.3, 468.2/459.0, 394.4/175.1, and 398.1/179.4 were selected for cortisol, cortisol-d_4, DHEA, and DHEA-d_4, respectively.

A C/D ratio was calculated based on the values of saliva cortisol and DHEA for each subject.

3.6.3 Statistical analysis

Repeated-measures analysis of variance (ANOVA) was employed to examine the diurnal cycles of saliva cortisol, DHEA, and C/D ratio across the three measurement times. Between-subject factors were the effort score (high or low), reward score (high or low), E/R ratio (high or low), OC score (high or low), and combination of E/R ratio and OC score (high, intermediate, or low). A series of measurement times (9:00hr, 12:00hr, and 15:00hr) was fit in as a within-subject factor. For each between-subject factor, main effects of the within- and between-subject factors and interaction between the within- and between-subject factors on the mean of

saliva cortisol, DHEA, and C/D ratio were examined. The Greenhouse-Geisser correction of degrees of freedom was applied where appropriate. The level of significance was 0.05 for all tests. Calculations were performed using SPSS 20 Japanese version for Windows (SPSS Japan Inc., Tokyo, Japan).

3.7 Ethical considerations

The original prospective cohort study was approved by the Ethics Committees of Fujita Health University, Japan before it was in practice. The present research protocol was also approved by the Research Ethics Committee of the Faculty of Public Health, Mahidol University.

The subjects made their decision by themselves on the participation in the present study. Before the data collection, the author visited the nursery schools to explain the purpose and methods of the study. All subjects gave their written consent to participate in the present study. The data used for the present study will be destroyed after the study ends.

CHAPTER IV
RESULTS

4.1 General characteristics

A total of 115 volunteers from 29 nursery schools who met the inclusion and exclusion criteria were analyzed. The baseline characteristics of the subjects are summarized in Table 4.1. Nearly 60% of the subjects were aged 20-29, while the remaining subjects were almost half split into the age groups 30-39 and 40-49. Nearly three-fourth of the subjects was employed as regular staff. There were a very small number of current smokers (2%). 10% of the subjects were in the ovulatory phase. More than one-fifth of the subjects complained of menstruation irregularity. For each of upper extremity and back symptoms, 4% of the subjects complained of severe symptoms. The remaining were dichotomized nearly on halves into the two groups of having none and moderate symptoms. 13% of the subjects went to a dentist for treatment of dental and gum diseases.

4.2 Job stress

The score range, median score, and number (percentage) of the subject with a high or a low score are indicated in Table 4.2 for each of the job stress indicators: effort, reward, E/R ratio, and overcommitment to work. For each of the job stress factors, the subjects were dichotomized almost on halves into the high and low score groups by employing the median score as the cut-off point.

Table 4.1 General characteristics of the 115 female nursery school teachers

Variables	Number (Percentage)
Age (years)	
20-29	66 (57)
30-39	26 (23)
40-49	23 (20)
Mean (SD)	30.8 (8.5)
Range	20 – 49
Employment status	
Regular staff	85 (74)
Contract workers	30 (26)
Current smoker	2 (2)
Ovulatory phase	12 (10)
Menstruation irregularity	25 (22)
Health problems	
Musculoskeletal disorders	
Upper extremity symptoms	
None	58 (50)
Moderate	52 (45)
Severe	5 (4)
Back symptoms	
None	54 (47)
Moderate	56 (49)
Severe	5 (4)
Dental and gum diseases	15 (13)
Other health problems	8 (7)

Table 4.3 indicates the number (percentage) of the subjects with a high, intermediate, and low level of combination of E/R ratio and OC score. The subjects were classified almost equally into the three groups.

Table 4.2 Job stress factors among the subjects

	Score		Number of the subjects (%)	
	Median	Range	High[1)]	Low[2)]
Effort	14	6 – 29	56 (49)	59 (51)
Reward	50	31 – 55	66 (57)	49 (43)
E/R ratio	0.529	0.233 – 1.351	57 (50)	58 (50)
Overcommitment to work	15	8 – 24	54 (47)	61 (53)

1) > median score

2) ≤ median score

Table 4.3 Combination of E/R ratio and OC score

Combination of E/R ratio and OC score	Number (%)
High[1)]	36 (31)
Intermediate[2)]	39 (34)
Low[3)]	40 (35)

1) High E/R ratio + high OC score

2) High E/R ratio + low OC score; or low E/R ratio + high OC score

3) Low E/R ratio + low OC score

Table 4.4 Diurnal cycle of saliva cortisol, DHEA, and C/D ratio

	Mean (SD)		
	9:00hr	12:00hr	15:00hr
Cortisol (nmol/l)	3.7 (3.1)	2.0 (1.6)	1.9 (1.0)
DHEA (nmol/l)	0.23 (0.18)	0.28 (0.20)	0.21 (0.15)
C/D ratio	19.9 (20.0)	8.9 (8.1)	11.3 (8.5)

4.3Diurnal cycle of saliva cortisol, DHEA, and C/D ratio

Table 4.4 indicates mean (SD) of saliva cortisol, DHEA, and C/D ratio at 9:00hr, 12:00hr, and 15:00hr.

4.4Job stress and saliva cortisol

The effects of job stress on saliva cortisol are indicated at Table 4.5.

For all of the job stress indicators, measurement time showed a significant main effect on the mean of saliva cortisol ($p < 0.001$). Multiple comparison revealed that saliva cortisol at 12:00hr ($p < 0.001$) and 15:00hr ($p < 0.001$) showed significant difference from that at 9:00hr.

Any of the job stress indicators did not present a significant interaction by time or main effect on the mean of saliva cortisol. Effort did not present significant interaction by time (F (1.46, 165.2) = 0.105, $p = 0.838$) or main effect (F (1, 113) = 0.092, $p = 0.762$). Reward did not present significant interaction by time (F (1.46, 165.2) = 0.182, $p = 0.763$) or main effect (F (1, 113) = 0.289, $p = 0.592$). E/R ratio did not present significant interaction by time (F (1.46, 165.4) = 0.015, $p = 0.960$) or main effect (F (1, 113) = 0.171, $p = 0.680$). Overcommitment to work did not present significant interaction by time (F (1.46, 165.2) = 0.177, $p = 0.768$) or main effect (F (1, 113) < 0.001, $p = 0.998$). The combination of E/R ratio and OC score did not present significant interaction by time (F (2.94, 164.7) = 0.946, $p = 0.418$) or main effect (F (2, 112) = 0.116, $p = 0.891$).

Table 4.5 Effects of job stress on saliva cortisol

	Cortisol (nmol/l; mean (SD))			*p*-value (job stress × time)[1)]
	9:00hr	12:00hr	15:00hr	
Effort score				0.838
High	3.7 (3.4)	1.9 (1.5)*	1.9 (1.1)*	
Low	3.7 (2.7)	2.1 (1.6)*	1.8 (1.0)*	
Reward score				0.763
High	3.8 (3.1)	2.0 (1.7)*	1.9 (1.1)*	
Low	3.5 (3.1)	2.0 (1.4)*	1.8 (0.9)*	
E/R ratio				0.960
High	3.7 (3.4)	2.0 (1.5)*	1.8 (0.9)*	
Low	3.7 (2.8)	2.0 (1.7)*	1.9 (1.1)*	
OC score				0.768
High	3.8 (3.2)	2.1 (1.8)*	1.8 (0.9)*	
Low	3.6 (2.9)	2.0 (1.4)*	1.9 (1.1)*	
E/R ratio × OC score[2)]				0.418
High	3.5 (3.2)	2.1 (1.7)*	1.8 (0.9)*	
Intermediate	4.2 (3.4)	1.9 (1.6)*	1.7 (1.0)*	
Low	3.5 (2.5)	2.1 (1.5)*	2.0 (1.2)*	

1) P-value of interaction between each job stress indicator (between-subject factor) and time (within-subject factor) was calculated with repeated-measures analysis of variance.

2) Combination of E/R ratio and OC score

*$p < 0.001$ for the within-subject comparisons to the value at 9:00hr

4.5 Job stress and saliva DHEA

The effects of job stress on saliva DHEA are indicated at Table 4.6.

For all of the job stress indicators, measurement time showed a significant main effect on the mean of saliva DHEA ($p < 0.001$). Multiple comparison revealed significant

Table 4.6 Effects of job stress on saliva DHEA

	DHEA (nmol/l; mean (SD))			*p*-value (job stress × time)[1)]
	9:00hr	12:00hr	15:00hr	
Effort score				0.707
High	0.24 (0.22)	0.28 (0.17)*	0.23 (0.13)†	
Low	0.22 (0.14)	0.28 (0.22)*	0.20 (0.17)†	
Reward score				0.223
High	0.22 (0.13)	0.28 (0.20)*	0.22 (0.17)†	
Low	0.25 (0.23)	0.29 (0.19)*	0.21 (0.13)†	
E/R ratio				0.614
High	0.24 (0.22)	0.28 (0.17)*	0.22 (0.13)†	
Low	0.22 (0.14)	0.28 (0.22)*	0.21 (0.17)†	
OC score				0.355
High	0.24 (0.21)	0.27 (0.16)*	0.22 (0.13)†	
Low	0.22 (0.16)	0.29 (0.23)*	0.21 (0.17)†	
E/R ratio × OC score[2)]				0.486
High	0.26 (0.25)	0.29 (0.18)*	0.24 (0.15)†	
Intermediate	0.21 (0.12)	0.25 (0.15)*	0.18 (0.08)†	
Low	0.23 (0.16)	0.30 (0.26)*	0.22 (0.20)†	

1) P-value of interaction between each job stress indicator (between-subject factor) and time (within-subject factor) was calculated with repeated-measures analysis of variance.

2) Combination of E/R ratio and OC score

*$p \leq 0.003$ for the within-subject comparisons to the value at 9:00hr

†$p < 0.001$ for the within-subject comparisons to the value at 12:00hr

difference between saliva DHEA at 9:00hr and at 12:00hr ($p \leq 0.003$). There was also significant difference in saliva DHEA between 12:00hr and 15:00hr ($p < 0.001$).

Any of the job stress indicators did not present a significant interaction by time or main effect on the mean of saliva DHEA. Effort did not present significant

interaction by time (F (1.79, 201.8) = 0.312, p = 0.707) or main effect (F (1, 113) = 0.272, p = 0.603). Reward did not present significant interaction by time (F (1.78, 201.6) = 1.517, p = 0.223) or main effect (F (1, 113) = 0.136, p = 0.713). E/R ratio did not present significant interaction by time (F (1.79, 202.1) = 0.453, p = 0.614) or main effect (F (1, 113) = 0.220, p = 0.640). Overcommitment to work did not present significant interaction by time (F (1.79, 202.3) = 1.021, p = 0.355) or main effect (F (1, 113) < 0.001, p = 0.987). The combination of E/R ratio and OC score did not present significant interaction by time (F (3.58, 200.3) = 0.848, p = 0.486) or main effect (F (2, 112) = 0.884, p = 0.416).

4.6 Job stress and saliva C/D ratio

The effects of job stress on saliva C/D ratio are indicated at Table 4.7.

For all of the job stress indicators, measurement time showed a significant main effect on the mean of saliva C/D ratio (p < 0.001). Multiple comparison revealed that saliva C/D ratio at 12:00hr (p < 0.001) and 15:00hr (p < 0.001) showed significant difference from that at 9:00hr. There was also significant difference in saliva C/D ratio between 12:00hr and 15:00hr (p = 0.001).

Any of the job stress indicators did not present a significant interaction by time or main effect on the mean of saliva C/D ratio. Effort did not present significant interaction by time (F (1.23, 138.6) = 0.783, p = 0.402) or main effect (F (1, 113) = 0.184, p = 0.669). Reward did not present significant interaction by time (F (1.23, 139.4) = 0.915, p = 0.360) or main effect (F (1, 113) < 0.001, p = 0.994). E/R ratio did not present significant interaction by time (F (1.23, 138.9) = 0.368, p = 0.589) or main effect (F (1, 113) = 0.096, p = 0.758). Overcommitment to work did not present significant interaction by time (F (1.23, 139.1) = 0.137, p = 0.764) or main effect (F

Table 4.7 Effects of job stress on saliva C/D ratio

	C/D ratio (mean (SD))			*p*-value (job stress × time)[1)]
	9:00hr	12:00hr	15:00hr	
Effort score				0.402
High	21.1 (26.2)	9.5 (8.4)*	10.8 (8.7)*†	
Low	18.8 (11.4)	8.4 (7.8)*	11.7 (8.2)*†	
Reward score				0.360
High	20.8 (21.9)	8.4 (6.5)*	10.8 (7.0)*†	
Low	18.7 (17.2)	9.6 (10.0)*	11.8 (10.1)*†	
E/R ratio				0.589
High	20.7 (25.7)	9.4 (9.8)*	11.0 (9.6)*†	
Low	19.1 (12.1)	8.5 (6.2)*	11.6 (7.2)*†	
OC score				0.764
High	20.2 (19.7)	8.9 (7.8)*	10.8 (8.4)*†	
Low	19.7 (20.3)	8.9 (8.5)*	11.7 (8.6)*†	
E/R ratio × OC score[2)]				0.449
High	19.1 (21.7)	9.2 (8.6)*	10.3 (8.5)*†	
Intermediate	22.9 (25.4)	9.0 (9.3)*	11.9 (10.0)*†	
Low	17.7 (10.1)	8.6 (6.4)*	11.5 (6.7)*†	

1) P-value of interaction between each job stress indicator (between-subject factor) and time (within-subject factor) was calculated with repeated-measures analysis of variance.

2) Combination of E/R ratio and OC score

*$p < 0.001$ for the within-subject comparisons to the value at 9:00hr

†$p = 0.001$ for the within-subject comparisons to the value at 12:00hr

(1,113) = 0.004, $p = 0.948$). The combination of E/R ratio and OC score did not present significant interaction by time (F (2.46, 137.9) = 0.851, $p = 0.449$) or main effect (F (2, 112) = 0.411, $p = 0.664$).

CHAPTER V
DISCUSSION

The present findings do not support the hypothesis that there are associations between job stress evaluated with the ERI model and saliva cortisol, DHEA, and C/D ratio. None of the job stress indicators, i.e., effort, reward, effort-reward imbalance, overcommitment to work, and combination of effort-reward imbalance and overcommitment to work, showed significant impacts on the diurnal cycles of saliva cortisol, DHEA, or C/D ratio. Daytime saliva cortisol, DHEA, and C/D ratio may not work as the biochemical indicators in evaluating job stress.

5.1 Job stress and saliva cortisol, DHEA, and C/D ratio

There are contradictory findings on the effects of job stress evaluated with the ERI model on saliva cortisol[21-25]. The present findings are concordant with the references reporting that effort-reward imbalance[23-25] and overcommitment to work[21, 22, 24] did not have significant impacts on daytime saliva cortisol concentration. To my knowledge, few researchers have not explored so far whether effort-reward imbalance and overcommitment to work are related to saliva DHEA and C/D ratio. The present study revealed the insignificant association between job stress evaluated with the ERI model and saliva DHEA and C/D ratio, adding new findings to the literature regarding the effects of job stress on the HPA activity more in detail than previous studies.

Acute stress often has enough impacts to cause health problems by a one-shot exposure[9], while job stress is too weak to develop health problems by a

one-shot exposure. In general, people have been exposed to job stress for a long time before health problems arise. There would be some difference between acute and job stress in the effects on the HPA axis. Even though job stress has some effects on the HPA activity, the effects would be different from those of acute stress. A unique mechanism regarding the relationship between prolonged stress like job stress and cortisol secretion is advocated[22, 29]: an initially heightened cortisol stress response occurs at the early stages of exposure to effort-reward imbalance, while the heightened cortisol stress response becomes weakened at the later stages by the down-regulation of the regulatory receptors or by means of enhanced tissue sensitivity. This mechanism could account for the present findings if the subjects in the present study who complained of job stress had suffered from the stress for a long time.

Job stress level to which the subjects were exposed must be carefully considered when interpreting the insignificant associations between job stress and saliva cortisol, DHEA, and C/D ratio in the present study. The present findings might be attributable to the low level of job stress to which the subjects were exposed. There are many ways to define those exposed to effort-reward imbalance. One usual method is that those who have an E/R ratio of 1.0 or greater are regarded as exposed to effort-reward imbalance. If this definition was applied to the present study, only 4 % of the subjects were regarded as exposed to effort-reward imbalance. Meanwhile, in another study where this definition was applied[30], 9.4% of the employees in a corporate manufacturing group in Japan were defined as exposed to effort-reward imbalance. According to the normative data by Siegrist[2], the developer of the ERI model, between 10 and 40 % of employees are supposed to be exposed to effort-reward imbalance. It is possible that the associations between job stress and saliva cortisol, DHEA, and C/D ratio might be prominent among those with more severe job stress. More extremely stressful situations may be needed to enhance the

HPA activity. In order to generalize the present findings, further examinations must be performed for those exposed to more severe job stress.

Differences in gender roles could account for the insignificant association between job stress evaluated with the ERI model and saliva cortisol, DHEA, and C/D ratio. The Charter of Work-Life Balance issued by the Cabinet Office, Japan[31] pointed out some issues of Japanese work customs: for example, working wives get exhausted by family matters because their husbands are involved into work too excessively to take care of their families. During off-work periods, the present subjects, i.e., female nursery school teachers, might face to other stressful situations that could stimulate the HPA activity. This might result in the insignificant associations between job stress evaluated with the ERI model and saliva cortisol, DHEA, and C/D ratio.

The subjects were volunteers who expressed an understanding to the purpose of the present study. It may mean that they were more interested in health than the others. The subjects might take any preventive measures against job stress and its consequences more frequently than the others. This could diminish the impacts of job stress evaluated with the ERI model on saliva cortisol, DHEA, and C/D ratio.

5.2Study limitations

Sampling bias could exist in this study. The subjects were volunteers enrolled only from healthy female nursery school teachers in 29 nursery schools. Moreover, the sample size (n = 115) might be small. As mentioned in the discussion, the subjects may be more interested in health than the others. Hence, the subjects may not be the representative of female employees in Japan, limiting the generalizability of the present findings.

The subjects were enrolled from 29 nursery schools, while the total

number of the subjects was 115. The sample size could be too small to detect the significant relationship between job stress and saliva cortisol, DHEA, and C/D ratio. Moreover, the analysis by nursery school is difficult due to the small statistic power.

The subjects only included the female aged between 20 and 49. It is unclear whether the present findings can be applied to the male and older female employees.

Saliva collection to measure cortisol and DHEA was carried out only in a working day although human physical responses to stress may vary day by day. In order to prove the reproducibility of the present findings, it would be necessary for the future studies to collect saliva in two or more working days.

Since the subjects were collected their saliva only daytime, the study does not answer to the question whether job stress has any impacts on awakening cortisol response (ACR). ACR is a series of phenomena that cortisol concentration rises up soon after the wake-up, peaks at about 60 min after the wake-up, and rapidly declines in a couple of hours. Cortisol in saliva collected during that period could be a better biochemical indicator to objectively evaluate job stress than that collected daytime. Eller et al.[21] found that female employees annoyed with effort-reward imbalance showed significantly higher saliva cortisol levels between the wake-up and 60 min after the wake-up than those not annoyed with effort-reward imbalance, while the difference in saliva cortisol levels became small 8 hours after the wake-up and at 18:00hr. Future studies are expected to collect saliva not only daytime but at the wake-up and over the following two to three hours.

Another job stress scale may provide different findings regarding the relationship between job stress and saliva cortisol, DHEA, and C/D ratio. There are some other job stress scales whose purposes are different from the ERI model. For example, the demand-control-support model[32] that has been widely used as the

representative job stress scale focuses exclusively on job task profiles, while the ERI model focuses on both situational and personal conditions at work.

CHAPTER VI
CONCLUSION AND RECOMMENDATION

The present findings did not show significant impacts of job stress evaluated with the ERI model on saliva cortisol, DHEA, or C/D ratio. Daytime saliva cortisol, DHEA, and C/D ratio may not work as biochemical indicators in evaluating job stress. Since the present study had some limitations, further studies are necessary to determine the usefulness of saliva cortisol, DHEA, and C/D ratio in evaluating job stress objectively.

6.1 Conclusion

A total of 115 nursery school teachers were enrolled in the present study to examine whether saliva cortisol, DHEA, and C/D ratio work as the biochemical indicator of job stress. With use of the ERI model, job stress of the subjects was evaluated through the following five profiles: effort, reward, E/R ratio, overcommitment to work, and combination of E/R ratio and overcommitment to work. The subjects provided saliva at 9:00hr, 12:00hr, and 15:00 in a working day. Saliva cortisol and DHEA were measured with LC-MS/MS method. Repeated-measured ANOVA was employed to clarify (1) whether exposure to job stress increases saliva cortisol concentration at daytime among the female nursery school teachers; (2) whether exposure to job stress decreases saliva DHEA concentration at daytime among the female nursery school teachers; and (3) whether exposure to job stress increases saliva cortisol/DHEA ratio (C/D ratio) at daytime among female nursery school teachers. Between-subject factors were the effort score (high or low), reward

score (high or low), E/R ratio (high or low), OC score (high or low), and combination of E/R ratio and OC score (high, intermediate, or low). A series of measurement times (9:00hr, 12:00hr, and 15:00hr) was fit in as a within-subject factor. As a result, all the job stress indicators showed insignificant interaction by time ($p > 0.05$) and main effect ($p > 0.05$) on saliva cortisol, DHEA, or C/D ratio. That is, the present finding did not show any significant association between job stress evaluated with the ERI model and saliva cortisol, DHEA, or C/D ratio. There are many possible ways of interpreting the findings as follows: The present findings may support the idea that cortisol stress response may be heightened at the early stages of exposure to job stress but weakened at the later stages; Since most of the subjects were not exposed to severe level of job stress, the association between job stress evaluated with the ERI model and saliva cortisol, DHEA, or C/D ratio was not prominent; Stress caused by off-work factors might increase cortisol secretion and seemingly decrease the impacts of job stress on saliva cortisol and DHEA; The subjects might take preventive ways against job stress and its consequences, which decrease cortisol stress response. The present study has the following limitations: Sampling bias might exist; The sample size was small; Saliva was collected only in a working day although human stress response may vary day by day; The present study did not provide answer to the question whether job stress has any impact on ACR; Employing another job stress model may produce different findings. Finally, the present research did not support the hypothesis that saliva cortisol, DHEA, and C/D ratio are the biochemical indicators to evaluate job stress. However, because of the above-written limitations, further studies must be carried out to determine the usefulness of saliva cortisol, DHEA, and C/D ratio as the biochemical indicators to evaluate job stress objectively.

6.2 Recommendation

The present finding could possibly mean that saliva cortisol, DHEA, and C/D ratio are not the biochemical indicators to evaluate job stress. However, this interpretation might not be justified because of the limitations that the present study possessed. Therefore, further studies are needed to more examine the usability of saliva cortisol, DHEA, and C/D ratio as the biochemical indicators to evaluate job stress objectively. The future research must take the following measures to get over the limitations of the present research:

- Sampling bias: To secure the representativeness of the working populations, the future researchers must increase the participation rate of eligible participants to the study. In addition they must collect as many subjects and address as many worksites as possible.
- Statistic power: The sample size must be as big as the future researchers can collect.
- Saliva collection: It is recommended to collect saliva in two or more working days to consider the variation of human stress response that could vary day by day. The future researcher must collect saliva at the wake-up and over the following two to three hours to examine the effects of job stress on ACR.
- Job stress scale: Another job stress scale could deserve to be employed in the future research.

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APPENDICES

APPENDIX A

QUESTIONNAIRE (ENGLISH)

Q1. How old are you now? (years old)

Q2. What is your employment status?

1. Regular employee working 8 hours a day
2. Temporary employee working 8 hours a day
3. Temporary employee working less than 8 hours a day

Q3. Are you a current smoker?

1. No
2. Yes (**Please specify the age at which you start smoking and the number of cigarettes you smoke a day:**)

Q4. Does your menstruation start regularly?

1. No
2. Yes (**Please specify the length menstruation cycle in day and the day at which your last menstruation started:**)

Q5. During the last week, did you feel any symptoms in your arms, shoulders, or hands?

1. No.
2. Yes. But, I did not care about it at all.
3. Yes. I cared about it. But, I did not need any support or treatment.
4. Yes. I needed support by my family/coworkers.
5. Yes. I needed medical treatment. (Check this if you were already in medical treatment.)

Q6. During the last week, did you feel any symptoms in your back?

1. No.
2. Yes. But, I did not care about it at all.
3. Yes. I cared about it. But, I did not need any support or treatment.
4. Yes. I needed support by my family/coworkers.
5. Yes. I needed medical treatment. (Check this if you were already in medical treatment.)

Q7. At present, are you regularly taking medication for any dental or gum diseases such as caries, periodontal diseases, etc.?

1. No
2. Yes (**Please specify the name of disease the doctor has diagnosed and medicine you are taking:**)

Q8. At present, are you regularly going for treatment for or taking medicine for any other diseases?

1. No
2. Yes (**Please specify the name of disease the doctor has diagnosed and medicine you are taking:**)

Q9. For each of the following statements, please indicate whether you agree or disagree with it. If you agree (questions 1-6, 10-13) or disagree (questions 7-9, 14-17) please also indicate how much you are generally distressed by this situation (1 = I am not at all distressed; 2 = I am somewhat distressed; 3 = I am distressed; 4 = I am very distressed).

eq1.I have constant time pressure due to a heavy work load.	Agree	1	2	3	4
	Disagree				
eq2.I have many interruptions and disturbances in my job.	Agree	1	2	3	4
	Disagree				
eq3.I have a lot of responsibility in my job.	Agree	1	2	3	4
	Disagree				
eq4.I am often pressured to work overtime.	Agree	1	2	3	4
	Disagree				
eq5.My job is physically demanding.	Agree	1	2	3	4
	Disagree				
eq6.Over the past years, my job has become more and more demanding.	Agree	1	2	3	4
	Disagree				

rq1.I receive the respect I deserve from my superiors.	Agree				
	Disagree	1	2	3	4
rq2.I receive the respect I deserve from my colleagues.	Agree				
	Disagree	1	2	3	4
rq3.I experience adequate support in difficult situations.	Agree				
	Disagree	1	2	3	4
rq4.I am treated unfairly at work.	Agree	1	2	3	4
	Disagree				
rq5.I have experienced or I expect to experience an undesirable change in my work situation.	Agree	1	2	3	4
	Disagree				
rq6.My job promotion prospects are poor.	Agree	1	2	3	4
	Disagree				
rq7.My job security is poor.	Agree	1	2	3	4
	Disagree				
rq8.My current occupational position adequately reflects my education and training.	Agree				
	Disagree	1	2	3	4
rq9.Considering all my efforts and achievements, I receive the respect and prestige I deserve at work.	Agree				
	Disagree	1	2	3	4
rq10. Considering all my efforts and achievements, my work prospects are adequate.	Agree				
	Disagree	1	2	3	4
rq11. Considering all my efforts and achievements, my salary/income is adequate.	Agree				
	Disagree	1	2	3	4

Q10. Please indicate to what extent (1 = strongly disagree; 2 = disagree; 3 = agree; 4 = strongly agree) you personally agree or disagree with these statements.

oq1. I get easily overwhelmed by time pressures at work.	1	2	3	4
oq2. I start thinking about work problems as soon as I get up in the morning.	1	2	3	4
oq3. When I get home, I can easily relax and forget all about work.	1	2	3	4
oq4. People close to me say I sacrifice too much for my job.	1	2	3	4
oq5. Work is usually still on my mind when I go to bed.	1	2	3	4
oq6. If I put off something that needs to be done today, I'll have trouble sleeping at night.	1	2	3	4

APPENDIX B

QUESTIONNAIRE (JAPANESE)

質問 1.

年齢はおいくつですか？ （＿＿＿＿＿）歳

質問 2.

あなたの雇用形態にもっとも近いものを 1 つだけ選んでください。

1. 正規職員　2. フルタイムの臨時社員　3. パートタイムの臨時社員

質問 3.

タバコを吸っていますか？

1. いいえ　　2. はい

⇓（**2. はい　を選んだ方はお答えください**）

- **タバコを吸いはじめたのは何歳のときですか？**（＿＿＿＿）歳
- **1 日にタバコを何本吸いますか？**　1 日あたり（＿＿＿＿）本

（唾液中のストレスホルモン量に影響を与える可能性があるため、おたずねします）

質問 4.

生理は定期的に来ますか？

1. いいえ　　2. はい

⇓（2. はい　を選んだ方はお答えください）

- **何日ごとに来ますか（＿＿＿＿＿＿＿＿）日**
- **最終月経日はいつですか？　（＿＿＿＿）月（＿＿＿＿）日**

質問 5.

先週 1 週間、腕・肩・手に何らかの症状はありましたか。もっとも近いものを 1 つだけ○で囲んで下さい。

1. 症状はない
2. 症状はあるが、気にするほどではない
3. 症状があり気になるが、そのままにしている
4. そのままにしておけないので誰かに相談しようと思う
5. 治療を必要とするほど症状が強く、仕事に差しつかえる（すでに治療中の人はここに○をつけて下さい）

質問 6.

先週 1 週間、腰・背中に何らかの症状はありましたか。もっとも近いものを 1 つだけ〇で囲んで下さい。

1. 症状はない
2. 症状はあるが、気にするほどではない
3. 症状があり気になるが、そのままにしている
4. そのままにしておけないので誰かに相談しようと思う
5. 治療を必要とするほど症状が強く、仕事に差しつかえる（すでに治療中の人はここに〇をつけて下さい）

質問 7.

現在、歯や口腔内の病気（虫歯、歯周病、その他）で治療（定期的な通院や服薬）を受けていますか？

1. いいえ　　2. はい

質問 8.

現在、その他の病気やケガで治療（定期的な通院や服薬）を受けていますか？

1. いいえ　　2. はい

⇓ **（2. はい　を選んだ方はお答えください）**

- 病名（＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿）

- 飲んでいる薬（＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿）

(職場環境についておたずねします)

(仕事内容とそれに対する考えをおたずねします)

質問 9.

以下の各文について、あなたにあてはまるか否か、まず□にチェックしてください。次に、□の右側に矢印（⇒⇒）があるときは、もっとも近いものを **1** つだけ○で囲んで下さい。（最近 **1** ヶ月の状況を考えてお答えください。）

		全く悩んでいない	いくらか悩んでいる	かなり悩んでいる	非常に悩んでいる
eq1.仕事の負担が重く、常に時間に追われている	は　い□⇒	**1**	**2**	**3**	**4**
	いいえ□				
eq2.邪魔が入って、中断させられることの多い　仕事だ	は　い□⇒	**1**	**2**	**3**	**4**
	いいえ□				
eq3.責任の重い仕事だ	は　い□⇒	**1**	**2**	**3**	**4**
	いいえ□				
eq4.しばしば、残業をせまられる	は　い□⇒	**1**	**2**	**3**	**4**
	いいえ□				
eq5.肉体的にきつい仕事だ	は　い□⇒	**1**	**2**	**3**	**4**
	いいえ□				
eq6.過去数年、だんだん仕事の負担が増えてきた	は　い□⇒	**1**	**2**	**3**	**4**
	いいえ□				
rq1.上司から、ふさわしい評価を受けている	は　い□				
	いいえ□⇒	**1**	**2**	**3**	**4**
rq2.同僚から、ふさわしい評価を受けている	は　い□				
	いいえ□⇒	**1**	**2**	**3**	**4**

rq3.困難な状況に直面すれば、同僚から充分な支援を受けられる	は　い□				
	いいえ□⇒	**1**	**2**	**3**	**4**
rq4.職場で公平に扱われていない	は　い□⇒	**1**	**2**	**3**	**4**
	いいえ□				
rq5.職場で好ましくない変化を経験している、もしくは今後そういう状況が起こりうる	は　い□⇒	**1**	**2**	**3**	**4**
	いいえ□				
rq6.昇進の見込みは少ない	は　い□⇒	**1**	**2**	**3**	**4**
	いいえ□				
rq7.失職の恐れがある	は　い□⇒	**1**	**2**	**3**	**4**
	いいえ□				
rq8.現在の職は、自分が受けた教育やトレーニングの程度を充分反映している	は　い□				
	いいえ□⇒	**1**	**2**	**3**	**4**
rq9.自分の努力と成果をすべて考えあわせると、 私は仕事上ふさわしい評価と人望を受けている	は　い□				
	いいえ□⇒	**1**	**2**	**3**	**4**
rq10. 自分の努力と成果をすべて考えあわせると、 私の将来の見通しはいい	は　い□				
	いいえ□⇒	**1**	**2**	**3**	**4**
rq11. 自分の努力と成果をすべて考えあわせると、 私のサラリー（収入）は適当だ	は　い□				
	いいえ□⇒	**1**	**2**	**3**	**4**

質問 10. 以下の各文について、もっとも近いものを **1** つだけ〇で囲んで下さい。

（最近 **1** ヶ月の状況を考えてお答えください。）

	全く違う	違う	そうだ	全くそうだ
oq1. 時間的なプレッシャーを感じやすい	**1**	**2**	**3**	**4**
oq2. 朝起きるとすぐ、仕事の問題を考え始める	**1**	**2**	**3**	**4**
oq3. 家に帰ると、すぐにリラックスでき、仕事のことをすべて　忘れてしまう	**1**	**2**	**3**	**4**
oq4. 私のことをよく知る人は、私は仕事のために自分を犠牲に　しすぎているという	**1**	**2**	**3**	**4**
oq5. 仕事のことが頭から離れず、寝床に入ってもそのことばかり考えている	**1**	**2**	**3**	**4**
oq6. 今日中にやるべきことをやむをえず明日に延ばさなければならないとしたら、夜眠れなくなる	**1**	**2**	**3**	**4**

APPENDIX C
DOCUMENTS OF ETHICAL CLEARANCE

Documentary Proof of Exemption
Ethical Review Committee for Human Research
Faculty of Public Health, Mahidol University

Protocol Title :	SALIVA CORTISOL AND DEHYDROEPIANDROSTERONE (DHEA) AS BIOCHEMICAL INDICATOR TO EVALUATE PSYCHOSOCIAL STRESS
Protocol No. :	281/2555
Principal Investigator :	Dr. Atsuhiko Ota
Affiliation :	Master of Public Health (International Program) Faculty of Public Health, Mahidol University

This protocol complies with a "Research with Exemption" category

Date of Issue : 17 December 2012

The aforementioned project have been reviewed and approved according to the Standard Operating Procedures of Ethical Review Committee for Human Research, Faculty of Public Health, Mahidol University.

S. Nanthamongkolchai

(Assoc. Prof. Sutham Nanthamongkolchai)
Chairman of Ethical Review Committee for Human Research

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疫学・臨床研究等倫理審査結果通知書

平成２０年３月２９日

藤田保健衛生大学医学部公衆衛生学教室

教授　小　野　雄一郎　殿

藤田保健衛生大学

医学部長　野　村　隆　英　印

下記研究について、以下のとおり決定しましたので通知いたします。

記

１．申請年月日　：　平成　２０　年　３　月　１７　日

２．申請者(代表)：　医学部・公衆衛生学教室・教授・小野雄一郎

３．研究区分　：　(疫学研究)　・　臨床研究　・　その他

４．審査区分　：　(新規)　・　変更

５．受付番号　：　０８－０４３

６．研究課題　：　「唾液中ストレスマーカーによるストレス評価および作業関連性筋骨格系障害との関連」

７．審査結果　：　承認する

８．備考　：

(Letter to the Chairperson of the Ethical Review Committee for Human Research, Faculty of Public Health, Mahidol University, to explain the Japanese-written notification of ethical approval the researcher obtained in Japan)

December 10, 2012

Chairperson of the Ethical Review Committee for Human Research
Faculty of Public Health, Mahidol University

To whom it may concern,

The Japanese-written notification attached to this document was issued by the Ethical Committee, Fujita Health University School of Medicine, Japan to provide the approval for the execution of the research entitled “Stress evaluated with saliva stress-related hormones and musculoskeletal disorders” with Prof. Dr. Yuichiro Ono and Senior Assist. Prof. Dr. Atsuhiko Ota, both of who belong to the Department of Public Health of the school.

Atsuhiko Ota (Researcher)
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