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The Effort-reward Imbalance work-stress model and daytime salivary cortisol and dehydroepiandrosterone (DHEA) among Japanese women

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We examined the influence of work-related effort–reward imbalance and overcommitment to work (OC), as derived from Siegrist's Effort–Reward Imbalance (ERI) model, on the hypothalamic–pituitary–adrenocortical (HPA) axis. We hypothesized that, among healthy workers, both cortisol and dehydroepiandrosterone (DHEA) secretion would be increased by effort–reward imbalance and OC and, as a result, cortisol-to-DHEA ratio (C/D ratio) would not differ by effort–reward imbalance or OC. The subjects were 115 healthy female nursery school teachers. Salivary cortisol, DHEA, and C/D ratio were used as indexes of HPA activity. Mixed-model analyses of variance revealed that neither the interaction between the ERI model indicators (i.e., effort, reward, effort-to-reward ratio, and OC) and the series of measurement times (9:00, 12:00, and 15:00) nor the main effect of the ERI model indicators was significant for daytime salivary cortisol, DHEA, or C/D ratio. Multiple linear regression analyses indicated that none of the ERI model indicators was significantly associated with area under the curve of daytime salivary cortisol, DHEA, or C/D ratio. We found that effort, reward, effort–reward imbalance, and OC had little influence on daytime variation patterns, levels, or amounts of salivary HPA-axis-related hormones. Thus, our hypotheses were not supported.

Epidemiological studies have indicated that work-related effort–reward imbalance and overcommitment to work (OC), as derived from Siegrist's Effort–Reward Imbalance (ERI) model[1], could cause various health problems, such as coronary heart disease[2–4], mental disorders[5,6], and sleep disorders[7–9]. In the ERI model, effort includes demands and obligation, while reward is distributed not only by money but by esteem, opportunities, and job security. Those who are rewarded insufficiently for their efforts are defined as being in a state of 'effort–reward imbalance' and are thereby exposed to stressful situations. OC is a personal trait related to dysfunctional coping methods in response to work-related stressful situations and feelings. It is characterised by attitudes, behaviors, and emotions that reflect excessive striving combined with a strong desire for approval and esteem.

The physiological mechanism underlying the association between the ERI model and health problems remains unclear. Acute exposure to a stressor stimulates the hypothalamic–pituitary–adrenocortical (HPA) axis and increases cortisol secretion[10]. Thus, researchers assumed that chronic exposure to stressful situations and feelings would induce similar responses. Accordingly, researchers have examined the influence of effort–reward imbalance and OC on patterns of variation, levels, and amount of salivary cortisol, which is considered an indicator of HPA activity that can be obtained non-invasively[11–22]. However, the influence of effort–reward imbalance and OC on daytime variation patterns, levels, and amount of salivary cortisol remains unclear. The relevant findings have been inconsistent. Eller et al.[13] found that high effort and effort–reward imbalance increases daytime salivary cortisol levels only in men. In contrast, Maina et al.[17] reported that high effort, low reward, and effort–reward imbalance were associated with decreased daytime salivary cortisol levels. Liao et al.[22] found a flatter diurnal cortisol decline associated with low reward and high effort–reward imbalance. Null associations between effort–reward imbalance and daytime salivary cortisol levels have also been reported[11,12,14]. Some research groups found a

significant positive association between OC and daytime salivary cortisol levels only in men[12,13]. Maina et al.[17] reported a positive but weak association between OC and daytime salivary cortisol amounts.

Measuring dehydroepiandrosterone (DHEA) and cortisol-to-DHEA ratio (C/D ratio) in addition to cortisol would offer scientifically interesting suggestions for examining the influence of effort–reward imbalance and OC on the HPA axis. DHEA is produced in the adrenal cortex and has a variety of physiological functions, including anti-glucocorticoid (i.e., anti-cortisol) effects[23]. Research on the physiological function of elderly people suggests that measuring cortisol alone is an oversimplification that results in an incomplete estimate of hypercortisolemia[24]. Some brain cells (e.g., hippocampal cells) and the immune system (i.e., lymphocytes) become more vulnerable to the cytotoxic and modulatory effects of glucocorticoids with age. A decline in DHEA secretion with age is a probable explanation for that degenerative change[24–26]. Hypercortisolemia may not be harmful if DHEA simultaneously exists at a sufficiently high level. Without considering the associations of effort–reward imbalance and OC with DHEA and C/D ratio, misleading explanations might be made on whether excessive cortisol secretion, which could be induced by effort–reward imbalance and OC, is indeed a health risk. To our knowledge, few relevant studies have assessed daytime DHEA or C/D ratio.

To clarify the influence of effort–reward imbalance and OC on the HPA axis, the question whether the daytime variation patterns, levels, and amount of salivary cortisol, DHEA, and C/D ratio differ by effort–reward imbalance and OC must be addressed. We examined this question under the hypotheses that, among healthy workers, both cortisol and DHEA secretion would be increased by effort–reward imbalance and OC and that, as a result, C/D ratio would not differ by effort–reward imbalance or OC. We cross-sectionally analysed the baseline dataset of a prospective cohort study that was designed to examine the relationship between psychosocial work-related factors and musculoskeletal disorders among nursery school teachers in Japan. Musculoskeletal disorders are highly prevalent among this population[27]. Adverse psychosocial work-related factors have been postulated as a possible cause[27].

Results

The subjects were 115 healthy female nursery school teachers in Japan. They were aged 20–49 years old, were not pregnant, and consented to participate in the present study. General participant characteristics (i.e., age, employment status, current smoker status, menstrual irregularity, ovulatory phase, and health disorders) and the ERI model indicators (i.e., effort score, reward score, effort-to-reward score ratio [E/R ratio], and OC score) are summarised in Tables 1 and 2, respectively.

Tables 3, 4, and 5 indicate the associations between the ERI model indicators and daytime variation patterns and levels of salivary cortisol, DHEA, and C/D molar ratio, respectively. Mixed-model analyses of variance (ANOVAs) were used. The subjects were divided into two groups at the median of each ERI model indicator. Those with a high E/R ratio were considered to be in effort–reward imbalance. There were no significant differences in daytime variation patterns or levels of salivary cortisol, DHEA, or C/D molar ratio by effort, reward, E/R ratio, or OC. Mixed-model ANOVAs, into which age, employment status, current smoking, menstruation irregularity, ovulatory phase, musculoskeletal symptoms, dental and gum diseases, and other health problems were entered as covariates, revealed that the interaction between ERI model indicators (between-subject factor) and the series of measurement times (9:00, 12:00, and 15:00; within-subject factor) and the main effect of ERI model indicators were not significant for daytime salivary cortisol, DHEA, or C/D molar ratio. On the other hand, there was a significant main effect of measurement time on daytime salivary cortisol, DHEA, and C/D molar ratio, regardless of the ERI model indicators ($P < 0.001$).

Table 1 | General subject characteristics (n = 115)

Variables	n (%)
Age (years) (Mean (SD): 30.8 (8.5))	
20–29	66 (57)
30–39	26 (23)
40–49	23 (20)
Employment status	
Regular staff	85 (74)
Contract worker	30 (26)
Current smoker	2 (2)
Menstruation irregularity	25 (22)
Ovulatory phase	12 (10)
Health disorders	
Musculoskeletal symptoms	75 (65)
Dental and gum diseases	15 (13)
Other health problems	8 (7)

SD: standard deviation.

Multiple comparisons showed that salivary cortisol values at 12:00 and 15:00 were significantly lower than they were at 9:00. Salivary DHEA values at 9:00 and 15:00 were significantly lower than they were at 12:00. Salivary C/D molar ratios at 12:00 were significantly lower than they were at 9:00. In addition, salivary C/D molar ratios at 15:00 were significantly different from those at 9:00 and 12:00.

Table 6 shows the associations between the ERI model indicators and area under the curve with respect to ground (AUC_G)[28] for salivary cortisol, DHEA, and C/D molar ratio. The AUC_G is an estimate of the daytime secretion amounts of hormones that was calculated using hormone levels at 9:00, 12:00, and 15:00. Multiple linear regression analyses were used to compute standardized partial regression coefficients of effort score, reward score, E/R ratio, and OC score on AUC_G of daytime salivary cortisol, DHEA, and C/D ratio. None of the ERI indicators—effort score, reward score, E/R ratio, or OC score—was significantly associated with AUC_G of daytime salivary cortisol, DHEA, or C/D molar ratio. Age, employment status, current smoking, menstruation irregularity, ovulatory phase, musculoskeletal symptoms, dental and gum diseases, and other health problems were adjusted in the analyses.

Discussion

The present study did not find a significant influence of effort–reward imbalance or OC on daytime salivary cortisol, DHEA, or C/D ratio. None of effort, reward, E/R ratio, or OC facilitated significant differences in daytime variation patterns, levels, or amount of salivary cortisol, DHEA, or C/D ratio. The present results are concordant with previous findings indicating that effort–reward imbalance[11,12,14] and OC[12,13] are not associated with daytime salivary cortisol in women. We employed salivary DHEA and C/D ratio in addition to cortisol to evaluate HPA activity in greater detail in comparison to previous studies. Finally, the present findings did

Table 2 | The Effort–Reward Imbalance (ERI) model indicators of the subjects (n = 115)

ERI indicator	Score		n (%)	
	Mean (SD)	Median (Range)	High[1)]	Low[2)]
Effort	14.3 (4.7)	14 (6–29)	56 (49)	59 (51)
Reward	49.3 (5.1)	50 (31–55)	66 (57)	49 (43)
E/R ratio	0.545 (0.218)	0.529 (0.233–1.351)	57 (50)	58 (50)
OC	15.2 (3.1)	15 (8–24)	54 (47)	61 (53)

SD: standard deviation; E/R ratio: effort-to-reward ratio; OC: overcommitment to work.
[1)]Above the median.
[2)]Less than or equal to the median.

Table 3 | Age-adjusted mean (standard error) of salivary cortisol according to the Effort–Reward Imbalance (ERI) model indicators and time

	Cortisol (nmol/l) by time			Interaction (ERI indicator × time)		Main effect of ERI indicator	
ERI indicator	9:00	12:00*	15:00*	F (degrees of freedom)	P	F (degrees of freedom)	P
High effort				0.523 (1.80, 186.7)	0.574	2.681 (1, 104)	0.105
Absent	3.7 (0.4)	1.9 (0.2)	1.9 (0.1)				
Present	3.7 (0.4)	2.1 (0.2)	1.8 (0.1)				
Low reward				0.149 (1.79, 186.4)	0.840	0.501 (1, 104)	0.481
Absent	3.7 (0.4)	2.0 (0.2)	2.0 (0.1)				
Present	3.6 (0.4)	2.1 (0.2)	1.7 (0.1)				
High E/R ratio				0.056 (1.79, 186.5)	0.930	3.825 (1, 104)	0.053
Absent	3.7 (0.4)	2.1 (0.2)	1.9 (0.1)				
Present	3.7 (0.4)	2.0 (0.2)	1.8 (0.1)				
High OC				0.515 (1.79, 186.1)	0.578	0.146 (1, 104)	0.703
Absent	3.7 (0.4)	2.0 (0.2)	1.9 (0.1)				
Present	3.7 (0.4)	2.0 (0.2)	1.8 (0.1)				

E/R ratio: effort-to-reward ratio; OC: overcommitment to work. F- and P-values were computed with mixed-model analyses of variance, into which age, employment status, current smoking, menstruation irregularity, ovulatory phase, musculoskeletal symptoms, dental and gum diseases, and other health problems were entered as covariates.
*: $P < 0.001$ for the within-subject comparison to 9:00, regardless of the ERI model indicators (paired samples t-test with the Bonferroni correction).

not support our hypothesis that, among healthy workers, both cortisol and DHEA secretion would be increased by effort–reward imbalance and OC and, as a result, C/D ratio would not differ by effort–reward imbalance or OC.

A unique mechanism could explain the lack of an association between stressful situations and feelings and HPA-related hormones: an initially increased cortisol secretion occurs at the early stage of chronic stress exposure, followed by weakened cortisol secretion at the later stage due to down-regulation of the regulatory receptors or enhanced tissue sensitivity[17,29]. Acute stress exposure is often strong enough to cause health problems after a single exposure[10], while most work-related stressful situations and feelings are generally too weak to facilitate health problems after a single exposure. Thus, cortisol secretion and HPA activity might not be higher in employees who have been in effort–reward imbalance or have had OC for a long time but have not yet developed stress-related health problems.

The lack of an association between effort–reward imbalance and HPA activity in the present study might be attributable to the low severity of effort–reward imbalance among the studied subjects. The effect of effort–reward imbalance on the HPA axis might be prominent only among those who are in more severe effort–reward imbalance. Effort–reward imbalance is commonly defined by an E/R ratio of 1.0 or greater. If this definition is applied to the present study, only 4% of the subjects were in effort–reward imbalance. Meanwhile, in another study that applied this definition, 9.4% of employees in a corporate manufacturing group in Japan were defined as being in effort–reward imbalance[30]. According to Siegrist, the developer of the ERI model, 10–40% of employees are in effort–reward imbalance[1]. In order to generalize the present findings, further research must be conducted among individuals in a more severe state of effort–reward imbalance.

Discussion on the other features of the participants is necessary for a complete interpretation of the present findings. First, because all the subjects were female, social differences in gender roles could, to some extent, account for the lack of an association of effort–reward imbalance and OC with the HPA axis. For instance, some working wives in Japan are exhausted by family matters because their husbands are too involved with work to care for their family[31]. Even during off-work time, some of the studied subjects might have been exposed to stressors that enhanced their HPA activity. This might have weakened the effect of effort–reward imbalance and OC on the HPA axis. Second, the subjects were volunteers. They might be interested in health and taking preventive measures against stress and its consequences. This could also diminish the influence of effort–

Table 4 | Age-adjusted mean (standard error) of salivary dehydroepiandrosterone (DHEA) according to the Effort–Reward Imbalance (ERI) model indicators and time

	DHEA (nmol/l) by time			Interaction (ERI indicator × time)		Main effect of ERI indicator	
ERI indicator	9:00	12:00*	15:00†	F (degrees of freedom)	P	F (degrees of freedom)	P
High effort				0.625 (1.89, 196.1)	0.527	1.394 (1, 104)	0.240
Absent	0.22 (0.02)	0.28 (0.03)	0.20 (0.02)				
Present	0.24 (0.02)	0.28 (0.03)	0.23 (0.02)				
Low reward				1.279 (1.89, 196.3)	0.280	0.001 (1, 104)	0.970
Absent	0.21 (0.02)	0.27 (0.02)	0.22 (0.02)				
Present	0.26 (0.02)	0.29 (0.03)	0.21 (0.02)				
High E/R ratio				0.302 (1.89, 196.6)	0.728	0.245 (1, 104)	0.622
Absent	0.22 (0.02)	0.28 (0.03)	0.20 (0.02)				
Present	0.25 (0.02)	0.29 (0.03)	0.22 (0.02)				
High OC				1.253 (2, 208)	0.288	0.424 (1, 104)	0.517
Absent	0.23 (0.02)	0.29 (0.03)	0.21 (0.02)				
Present	0.24 (0.02)	0.27 (0.03)	0.22 (0.02)				

E/R ratio: effort-to-reward ratio; OC: overcommitment to work. F- and P-values were computed with mixed-model analyses of variance, into which age, employment status, current smoking, menstruation irregularity, ovulatory phase, musculoskeletal symptoms, dental and gum diseases, and other health problems were entered as covariates.
*: $P < 0.001$ for the within-subject comparisons to 9:00, regardless of the ERI model indicators (paired samples t-test with the Bonferroni correction).
†: $P < 0.001$ for the within-subject comparisons to 12:00, regardless of the ERI model indicators.

Table 5 | Age-adjusted mean (standard error) of salivary cortisol-to-dehydroepiandrosterone molar ratio (C/D ratio) according to the Effort–Reward Imbalance (ERI) model indicators and time

	C/D ratio by Time			Interaction (ERI indicator × time)		Main effect of ERI indicator	
ERI indicator	9:00	12:00*	15:00*†	F (degrees of freedom)	P	F (degrees of freedom)	P
High effort				0.933 (2, 208)	0.395	0.204 (1, 104)	0.652
Absent	18.8 (2.5)	8.4 (1.0)	11.7 (1.0)				
Present	21.1 (2.6)	9.5 (1.1)	10.8 (1.0)				
Low reward				0.434 (1.89, 196.5)	0.637	0.462 (1, 104)	0.498
Absent	21.2 (2.4)	8.6 (1.0)	11.1 (1.0)				
Present	18.1 (2.8)	9.4 (1.1)	11.4 (1.1)				
High E/R ratio				0.137 (1.89, 196.4)	0.861	1.777 (1, 104)	0.185
Absent	19.4 (2.6)	8.6 (1.0)	11.7 (1.0)				
Present	20.5 (2.6)	9.3 (1.0)	10.8 (1.0)				
High OC				0.650 (1.88, 195.8)	0.514	0.048 (1, 104)	0.827
Absent	19.5 (2.5)	8.9 (1.0)	11.6 (1.0)				
Present	20.4 (2.7)	9.0 (1.1)	10.9 (1.1)				

E/R ratio: effort-to-reward ratio; OC: overcommitment to work. F- and P-values were computed with mixed-model analyses of variance, into which age, employment status, current smoking, menstruation irregularity, ovulatory phase, musculoskeletal symptoms, dental and gum diseases, and other health problems were entered as covariates.
*: $P < 0.001$ for the within-subject comparisons to 9:00, regardless of the ERI model indicators (paired samples t-test with the Bonferroni correction).
†: $P < 0.001$ for the within-subject comparisons to 12:00, regardless of the ERI model indicators.

reward imbalance and OC on the HPA axis. At the same time, they might not be representative of female employees in Japan, limiting the generalizability of the present findings. Third, the subjects only included females aged between 20 and 49. It is unclear whether the present findings can be applied to males or older female employees. Finally, the sample size might have been insufficient to detect any significant influence of effort–reward imbalance and OC on the HPA axis. However, it was large enough to detect a significant time course of salivary hormone levels.

There were some limitations in our saliva collection method. Saliva was collected in a single working day. Although Edwards et al.[32] reported high reproducibility of area under the curve for daytime salivary cortisol across two consecutive days, human stress responses might vary according to the measurement day. In order to demonstrate the reproducibility of the present findings, future studies should collect saliva over at least two working days. Moreover, we only collected saliva samples at 9:00, 12:00, and 15:00. A more accurate estimate of daytime salivary cortisol and DHEA secretion may be obtained by collecting saliva more frequently. We were unable to evaluate cortisol awakening response (CAR), which is the change in cortisol concentration during the first hour after waking up[33,34]. Along with the daytime cortisol output, CAR is considered an estimate of HPA activity. CAR is independent from daytime cortisol output[32,35], which can be partly explained by genetic factors[36,37]. We do not believe that our findings were skewed by absent information on salivary hormones during the first hour after waking up. CAR is not yet an established indicator of HPA activity in response to effort–reward imbalance and OC. Researchers have reported inconsistent findings on the associations of effort–reward imbalance and OC with CAR. Eller et al.[13] reported that employees in effort–reward imbalance raised CAR in both men and women. Then, Almadi et al.[21] reported a similar finding among healthy male workers. Contrary to this, Maina et al.[17] reported a significant negative association between E/R ratio and CAR. Furthermore, some researchers have failed to find a significant association between effort–reward imbalance and CAR[18–20,22]. Steptoe et al.[12] and Eller et al.[13] addressed the influence of OC on CAR, finding that OC raised CAR, but only in men. Bathman et al.[20] reported a non-significant association between OC and CAR. So far, very few relevant studies have employed DHEA in addition to cortisol. In sum, it remains inconclusive whether effort–reward imbalance and OC affect cortisol, DHEA, and C/D ratio during the first hour after waking up. The influence of effort–reward imbalance and OC on cortisol, DHEA, and C/D ratio around wake-up time must be further investigated. This must contribute to determining the underlying physiological mechanism by which effort–reward imbalance and OC lead to health problems.

We did not adjust for some potential confounders in the association between the ERI model and the HPA axis, such as sleep-related information (awakening time, sleep duration, sleep quality, etc.), education level, and marital status. Absent sleep-related information, especially with regard to awakening time, might affect salivary cortisol levels at 9:00. All the studied subjects started working around 9:00 on the saliva collection day. We assumed that the majority of them woke up at 7:00 or earlier, although we did not confirm

Table 6 | Standardized partial regression coefficients (SPRCs) of the Effort–Reward Imbalance (ERI) model indicators on area under the curve with respect to ground (AUC_G) for daytime salivary cortisol, dehydroepiandrosterone (DHEA), and cortisol-to-DHEA molar ratio (C/D ratio)

	ERI model indicator (independent variables)							
	Effort score		Reward score		E/R ratio		OC score	
AUC_G (dependent variable)	SPRC	P	SPRC	P	SPRC	P	SPRC	P
Cortisol	−0.159	0.151	0.019	0.848	−0.112	0.304	−0.128	0.214
DHEA	−0.061	0.528	−0.002	0.978	−0.041	0.668	−0.049	0.585
C/D ratio	−0.095	0.380	0.038	0.699	−0.078	0.460	−0.080	0.428

E/R ratio: effort-to-reward ratio; OC: overcommitment to work. AUC_Gs were calculated using salivary hormone levels at 9:00, 12:00, and 15:00. SPRCs and P-values were computed with multiple regression analysis, in which age, employment status, current smoking, menstruation irregularity, ovulatory phase, musculoskeletal symptoms, dental and gum diseases, and other health problems were adjusted.

this. Thus, their salivary cortisol levels at 9:00 would be under little influence of CAR. Education level could be related to the way in which the subjects took preventive measures against stress. However, we did not assess education levels on account of the probable homogeneity. All subjects were nursery school teachers. In principle, they had to take a relevant 2-year professional education program after high school graduation to receive certification as nursery school teachers in Japan. We neither asked nor adjusted for marital status. We were concerned that inquiring about marital status might be a sensitive matter, potentially leading to unwanted stress. In addition, some unmarried subjects might have a boyfriend or be in a de facto state of marriage. We did not include pregnant women due to increased cortisol levels during the gestation period[38]. Therefore, we concluded that including marital status in the analysis is unnecessary.

Besides the ERI model, there are a variety of psychometric concepts to assess multifaceted psychosocial work environment and workers' mental states. Some examples include the Demand–Control–Support Model[39], organizational justice[40], and burnout syndrome[41]. Further research should consider these concepts to comprehensively understand the influence of the psychosocial work environment and workers' mental states on the HPA axis.

Methods

The present study was carried out in accordance with the Ethical Guidelines for Epidemiological Research established by the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare, Japan. The study was approved by the Ethics Review Committees of Fujita Health University, Japan (No. 11-098). One of the authors (A.O.) visited the nursery schools where the eligible subjects were working and explained the purpose and methods of the study before recruiting the subjects. All subjects gave their written consent for participation in the study.

The 115 subjects were recruited from 29 nursery schools in Aichi prefecture, Japan. The inclusion criteria were (1) aged between 20 and 49, (2) not pregnant, and (3) not taking medicine that could affect secretion of cortisol or DHEA, such as oestrogen, oral contraceptives, steroids, and antidepressants. The baseline data and saliva were collected in a working day from December through February of the years 2010–2012. Participants were asked to fill out a self-report questionnaire for general characteristics and the ERI model indicators and to provide saliva for measuring salivary cortisol and DHEA.

General characteristics included age, employment status (regular staff or contract worker), current smoking status, menstrual irregularity, ovulatory phase, and health disorders. Those who replied negatively to the question "Does your menstruation begin regularly?" were regarded as having menstrual irregularity. To determine whether the subjects without menstrual irregularity were in their ovulatory phase, they were asked about the length of the menstrual cycle and the day on which their last menstruation began. The half-length of the menstrual cycle was added to the beginning day of their last menstruation in order to estimate the ovulation day. The subjects were regarded as being in the ovulatory phase if the saliva collection day was within 3 days (both ways) of the estimated ovulation day. With regard to health disorders, subjects were asked whether they had any subjective symptoms of musculoskeletal disorders (upper extremities or back problems) or were in treatment for dental and gum diseases or any other health problems.

To score the ERI model indicators, the Japanese version of the ERI Questionnaire[42] was used. Items to evaluate effort (6 items) and reward (11 items) consisted of yes/no questions followed by 4-point Likert scales. Meanwhile, OC was assessed with 6 items using only a 4-point Likert scale. Among the studied subjects, the Cronbach's alpha coefficients for effort, reward, and OC were 0.84, 0.77, and 0.77, respectively. The questionnaire details and how to score the ERI model indicators have been described elsewhere[7,30,43].

Saliva was collected at 9:00, 12:00, and 15:00 on a working day. The subjects were asked to discharge 1 ml of saliva directly in a syringe at each collection time. We did not employ cotton collection devices, like Salivette, because of the possible interference on quantification of salivary DHEA. It has been reported that a significant correlation between serum and salivary DHEA can be lost when saliva is collected using such cotton devices[44,45]. The subjects rinsed their mouths with drinking water before each saliva collection. They did not eat for 30 minutes before each saliva collection. Saliva samples were kept in a freezer at −25°C soon after collection.

Aska Pharmaceutical Medical Co. Ltd. (Kawasaki, Japan) quantified salivary cortisol and DHEA with the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method as follows. The thawed saliva was centrifuged. The supernatant fluids were collected for analysis. As an internal standard, 1 ng of DHEA-[2, 2, 4, 6-$^2H_4$] (DHEA-d_4) and 1 ng of cortisol-[9, 11, 12, 12-$^2H_4$] (cortisol-d_4) were added to the saliva sample (1.0 ml). Steroids were extracted with 4 ml of ethyl acetate. The organic layer was separated and evaporated to dryness using a centrifugal evaporator. The residue was treated with 100 μl of a derivatizing reagent mixture (10 mg of 4-dimethylaminopyridine, 20 mg of 2-methyl-6-nitrobenzoic anhydride, and 25 mg of picolinic acid in 1 ml of tetrahydrofuran) and 20 μl of triethylamine. Then, the mixture was left at room temperature for 30 min. After diluting the reaction mixture with 1% acetic acid in 1 ml of water, the diluted mixture was applied to the SPE cartridge (Bond Elut C18). The cartridge was washed with 1 ml of water and 2 ml of 30% aqueous acetonitrile. The derivatives were eluted with 3 ml of 80% aqueous acetonitrile. After evaporation, the residue was reconstituted in 100 μl of 40% aqueous acetonitrile and was then injected into the LC-MS/MS system. The system consisted of an API-4000 triple-stage quadrupole mass spectrometer equipped with an ESI ion source (AB SCIEX, Framingham, MA, US) and an Agilent HPLC system (Agilent 1100, Agilent Technologies, Santa Clara, CA, US) with a HTC-PAL (CTC Analytics AG, Zwingen, Switzerland). The column was a Cadenza CD-C18 column (250 × 3 mm i.d., 3 μm, Imtakt, Kyoto, Japan) used at 40°C. The mobile phase consisted of 0.1% formic acid (Solvent A) and acetonitrile (Solvent B). The gradient elution of A : B was as follows: 50 : 50 to 40 : 60 (0–1.0 min), 40 : 60 to 10 : 90 (1.0–4.0 min), 10 : 90 to 0 : 100 (4.0–9.0 min), 0 : 100 (9.0–12.0 min), and 50 : 50 (12.0–15.0 min) at a flow rate of 0.4 ml/min. MS/MS conditions were as follows: spray voltage, 4,500 V; nitrogen collision gas, 45 psi; curtain gas, 11 psi; ion source temperature, 450°C; and ion polarity, positive. For detection using multiple reaction monitoring, ion transitions of m/z were 468.2/309.3 (cortisol), 472.2/454.0 (cortisol-d_4), 394.4/175.1 (DHEA), and 398.1/179.4 (DHEA-d_4).

For analysis, mixed-model ANOVAs and multiple linear regression analyses were applied. Mixed-model ANOVAs were conducted to examine whether daytime variation patterns and levels of salivary cortisol, DHEA, and C/D molar ratio differed by the ERI model indicators (i.e., effort, reward, E/R ratio, and OC). The between-subject factors were effort (high or low), reward (low or high), E/R ratio (high or low), and OC (high or low). The subjects were divided into two (high and low) groups at the median of each ERI model indicator in order to optimally equalize the number of subjects between the two groups and avoid reduction of statistical power. As we mentioned in the discussion, effort-reward imbalance is often defined by an E/R ratio of 1.0 or greater. However, we did not apply this definition to the studied subjects because only 4% of them corresponded to it. The within-subject factor was the series of measurement times (9:00, 12:00, and 15:00). The interactions between the between- and within-subject factors on salivary cortisol, DHEA, and C/D molar ratio were calculated to determine whether daytime variation patterns of the salivary hormones differed by the between-subject factors. The main effects of the between- and within-subject factors on the salivary HPA-related hormones were computed to determine whether the salivary hormone levels differed by those factors. F- and P-values were adjusted by the Greenhouse–Geisser correction for degrees of freedom for cases in which the sphericity assumption was violated. For the analyses, the values of salivary cortisol, DHEA, and C/D molar ratios were log-transformed for normality. Multiple linear regression analyses were conducted to calculate standardized partial regression coefficients of effort score, reward score, E/R ratio, and OC score (independent variables) on AUC_G of salivary cortisol, DHEA, and C/D ratio (dependent variables). AUC_G was calculated as proposed by Pruessner et al.[28] Age, employment status, current smoking, menstruation irregularity, ovulatory phase, musculoskeletal symptoms, dental and gum diseases, and other health problems were adjusted as potential confounders/moderators in both mixed-model ANOVAs and multiple linear regression analyses. The level of significance was 0.05 for all tests. Calculations were performed using IBM SPSS Statistics 20 Japanese version for Windows (IBM Japan, Tokyo, Japan).

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Author contributions

A.O., J.M. and Y.O. conceived the study design. A.O. and J.M. collected and analyzed the data. A.O., N.H., T.R., N.S. and H.Y. discussed the results. A.O. wrote the manuscript, on which all the other authors commented. A.O. and Y.O. obtained the grants for the present study.

Additional information

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