Cortisol and dehydroepiandrosterone (DHEA) are the main products of adrenergic activity in response to the adrenocorticotrophic hormone (ACTH). It is well documented that salivary cortisol levels increase and reach a peak 30-60 minutes after awakening.\(^1\)\(^-\)\(^3\) The act of awakening stimulates cortisol secretion,\(^4\) an event that is called the cortisol awakening response (CAR).\(^5\) Cortisol levels and CAR are established indices of stress responses that have been used in various fields of research.\(^6\) A steroid, 17\(^\alpha\)-hydroxypregnenolone, is a co-precursor for the biosynthesis of cortisol and DHEA in the adrenal cortex.\(^7\) DHEA has been described to function as an anti-glucocorticoid.\(^8\),\(^9\) Thus, the cortisol-to-DHEA ratio (C/D ratio) is accepted as an index of steroidogenic balance and is used as a reliable adrenocortical index of allostatic states,\(^10\) psychosomatic disorders,\(^11\)-\(^13\) and anxiety and negative mood.\(^14\)

Some investigations have observed no differences in the daytime cortisol levels between workdays and the weekend.\(^15\),\(^16\) However, other studies have shown differences in CAR between workdays and the weekend among working subjects.\(^17\)-\(^20\) These four studies have shown that CAR patterns are the same on

### INTRODUCTION

Cortisol and dehydroepiandrosterone (DHEA) are the main products of adrenocortical activity in response to the adrenocorticotrophic hormone (ACTH). It is well documented that salivary cortisol levels increase and reach a peak 30-60 minutes after awakening.\(^1\)\(^-\)\(^3\) The act of awakening stimulates cortisol secretion,\(^4\) an event that is called the cortisol awakening response (CAR).\(^5\) Cortisol levels and CAR are established indices of stress responses that have been used in various fields of research.\(^6\) A steroid, 17\(^\alpha\)-hydroxypregnenolone, is a co-precursor for the biosynthesis of cortisol and DHEA in the adrenal cortex.\(^7\) DHEA has been described to function as an anti-glucocorticoid.\(^8\),\(^9\) Thus, the cortisol-to-DHEA ratio (C/D ratio) is accepted as an index of steroidogenic balance and is used as a reliable adrenocortical index of allostatic states,\(^10\) psychosomatic disorders,\(^11\)-\(^13\) and anxiety and negative mood.\(^14\)

Some investigations have observed no differences in the daytime cortisol levels between workdays and the weekend.\(^15\),\(^16\) However, other studies have shown differences in CAR between workdays and the weekend among working subjects.\(^17\)-\(^20\) These four studies have shown that CAR patterns are the same on
workdays and on the weekend, but that CAR levels are more profound on weekdays than on the weekend. Differences in the slope of CAR between workdays and the weekend reflect the anticipation or preparation for a stressful work life in the approaching week. These may include anticipation of job-related stress or preparation of strategies for managing an upcoming task(s) that may act as a cognitive or internal stressor.17,18

Little information is available on the day-to-day psychological and psychiatric differences among employed individuals, but it is widely believed that Monday is the “worst day of the week” in terms of mood and well-being.21 Larsen and Kasimatis22 observed a weekly mood cycle, with the average ratings of depression being the lowest on Fridays and Saturdays and the highest on Mondays and Tuesdays. Furthermore, other studies have shown that the number of patients with myocardial infarctions varied within the week, with the number reaching a peak on Mondays.23,24 These results suggest that there may exist day-to-day differences in mood and physical or psychological stress, especially on the first or second day after transitioning from the weekend or public holiday activities among working individuals. However, the differences in cortisol levels and CAR between workdays and weekends were not enough to discriminate the physical or psychological stress between the first or second workday after transition from the weekend and other workdays. Different approaches are needed to explain the stress experienced by working individuals transitioning from the weekend and to elucidate the day-to-day differences in the stress between workdays.

The objectives of this study are to test the hypothesis that there are differences in the adrenocortical status between weekends and workdays, as well as between the first or second workday after transitioning from the weekend and the other workdays in working subjects. This hypothesis is based on the observation that working subjects usually perceived more stress on workdays than non-workdays, with Mondays having more stress than other workdays.25 In order to test our hypothesis, working individuals were recruited based on their job status, job tenure, education level, and age. The differences in adrenocortical secretory activities after the awakening period between workdays and the weekend, as well as the day-to-day differences in levels of cortisol, DHEA, and the molar C/D ratios at the time of maximal adrenocortical activities were investigated.

MATERIALS AND METHODS

Subjects

For this study, 74 full-time employed individuals working in the hospital were recruited (35 male and 39 female subjects). The mean age of the study subjects was 41.39 ± 10.22 years. Due to age-related decreases in DHEA levels,26 no participant over the age of 56 years of (ages ranged between 22-55 years old) was included. The subjects worked Monday through Friday from 9 a.m. to 6 p.m. and on Saturday from 9 a.m. to 4 p.m. All of the subjects had worked more than three months at the position they held at the time of this study, and none of the subjects were taking any type of medication. None of the study subjects had any pathological conditions such as diabetes, cancer, hypotension, or hypertension. The samples were collected between May and July of 2008. Among the 74 participants, more than one batch of samples was collected from some of the participants. Saliva samples were collected from 31 subjects for the preliminary experiment, and from 46 participants for the main objectives of this study.

Saliva collection

All participants were instructed to abstain from smoking, eating certain kinds of food items, and consuming caffeine-containing beverages. They were instructed to neither brush their teeth nor rinse their mouth with water before collecting the saliva samples. The participants were instructed to label the samples by providing information regarding the sampling time, date, and duration of sleep on the provided labels. A minimum volume of 2 mL of saliva was collected directly into a collecting tube by expectoration. We selected this collection method because cotton-based saliva collection is not adequate for the salivary immunoassay of DHEA.27 This study was conducted in accordance with the principles laid down by the Declaration of Helsinki. All participants provided their informed consent and were provided with information about their hormone levels. Ethical approval for this study was obtained from the local research ethics committee.

A batch of samples collected from a participant was simultaneously excluded when a saliva sample was missed or was collected outside of the designated time point (s) if discovered after the examination of the labels on each tube. In addition, a batch of samples was rejected if a sample was useless, reddish in color, or if the sample contained an insufficient volume to perform the steroid assays. In conclusion, we accepted saliva samples collected from 26 subjects for the preliminary experiment, and 28 subjects for the main objectives of the present study. General information on the saliva collection time and basic health of the 28 subjects who collected saliva samples for 7 consecutive days is summarized in Table 1. All saliva samples were analyzed at the Hormone Research Center at Chonnam National University.
Measurement of salivary cortisol and DHEA levels
Steroid levels in the saliva were determined using a radioimmunoassay (RIA), employing a previously described liquid-phase double-antibody method.26 Iodine-125-labeled cortisol [cortisol-3-(O-carboxymethyl) oximino-(2-[125I]iodohistamine)] was obtained from PerkinElmer Life and Analytical Sciences (MA, USA). Since iodine-125-labeled DHEA [DHEA-7-(O-carboxymethyl) oximino-(2-[125I]iodohistamine)] is not commercially available in Korea, it was prepared by modifying a previously described protocol for the radioiodination of steroids.28 Cortisol and DHEA antisera were purchased from Biogenesis (Oxford, UK). Cortisol antiserum cross-reacts with aldosterone (0.001% cross reactivity), 11-deoxycorticosterone (4.1%), 11-deoxycortisol (5.7%), 21-deoxycortisol (0.5%), corticosterone (1.2%), and other steroids (< 0.01%). DHEA antiserum cross-reacts with 5α-androstane-β, 17β-diol (6.3% cross reactivity), androstenedione (1.3%), testosterone (0.1%), and other steroids (< 0.05%). Multiple quality control samples were prepared, kept frozen, and used in each assay. The intra- and inter-assay coefficients of variation for cortisol were 7.34% (n = 20) and 8.28% (n = 9), respectively. The intra- and inter-assay coefficients of variation for DHEA were 6.90% (n = 20) and 8.28% (n = 7), respectively. The analytical sensitivities of the assay for cortisol and DHEA were 10 pg/mL and 1 pg/mL, respectively.

Statistical analyses
The results are presented as means ± standard deviations (SDs) and as medians and percentiles. Since cortisol and DHEA levels were repeatedly measured for seven consecutive days, the inter-subject variations were eliminated in determination of the day-to-day differences by using an analysis of variance (ANOVA) for a randomized complete block design (RCBD). The seven daily measurements of cortisol levels, DHEA levels, or the molar C/D ratios from each participant were considered to be a complete block. This complete block was used to determine the day-to-day differences,29 and a post-hoc test (Duncan’s multiple-range test) was performed to determine the significance of the day-to-day differences. Pearson’s correlation coefficients for the cortisol and DHEA levels, the molar C/D ratios for each day of the sample collection week, and the area under the curve (AUC) were calculated using GraphPad Prism version 5.01 for Windows (San Diego, CA, USA), and statistical calculations were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

Table 1. Characteristics of the Saliva Sample

|                        | Men (n = 15) | Women (n = 13) |
|------------------------|-------------|---------------|
| Age (yrs)              | 43.7 (10.9) | 40.5 (10.5)   |
| Marital status (% married) | 12 (80)    | 7 (54)        |
| Current smokers (%)    | 4 (27)      | 1 (7)         |
| Body mass index        | 23.8 (2.1)  | 23.4 (2.2)    |
| Blood pressure*        |             |               |
| Diastolic (mmHg)       | 75.9 (4.9)  | 69.2 (2.9)    |
| Systolic (mmHg)        | 121.1 (2.9) | 110.7 (1.2)   |
| Collection of samples after awakening on workday (m) | 28.9 (2.6) | 28.2 (2.8) |
| Collection of samples after awakening on Sunday (m) | 28.4 (2.5) | 28.2 (1.7) |
| Sleep duration on work day (h) | 6.78 (0.6) | 6.88 (0.7) |
| Sleep duration on Sunday (h) | 6.92 (0.4) | 7.33 (0.8) |

Values are given as means (standard deviations) and number (%) as appropriate.
*Mean blood pressure checked 3 times at 17:00-18:00 among workdays.

RESULTS

Background information
The cortisol levels at different periods after awakening (immediately upon awakening, 15, 30, and 45 minutes after awakening) were measured from saliva samples collected on workdays from 26 subjects (16 male and 12 female, aged 28-55 years old) (Fig. 1). Cortisol levels immediately upon awakening, 15, 30, and 45 minutes after awakening in males were 7.18 ± 3.06 nmol/L, 14.09 ± 6.68 nmol/L, 14.77 ± 7.89 nmol/L and 8.46 ± 4.88 nmol/L, respectively, and those in females were 8.49 ± 2.42 nmol/L, 14.08 ± 6.35 nmol/L, 15.69 ± 4.45 nmol/L and 10.45 ± 5.37 nmol/L, respectively. Because there were no differences in cortisol levels at each collection time between male and female ($p > 0.05$ in all analyses), the data was pooled together.

The mean cortisol levels were increased and reached a peak 30 minutes after awakening. The mean cortisol level, at its peak, was increased by 113.7 ± 76.96% (7.4 ± 5.6
The results of the preliminary study showed that the cortisol levels reached a peak 30 minutes after awakening. Thus, participants were asked to collect their saliva samples between 25-35 minutes after awakening and to continue collecting samples at the same time for 7 consecutive days.

Changes in cortisol levels from Monday through Sunday
Cortisol levels were measured in saliva samples collected from 28 subjects (15 male and 13 women, aged 24-53 year) for seven consecutive days at the designed time point (i.e., 30 ± 5 minutes after awakening from a normal nocturnal sleep) (Fig. 2).

The mean cortisol levels on Monday through Saturday were in the range of 13.67-14.94 nmol/L, and those on Sunday were 10.39 nmol/L (Fig. 2). The RCBD-ANOVA test revealed significant day-to-day differences in the mean cortisol levels between the days of the week (F6, 168 = 5.77, p < 0.01). Duncan’s post-hoc test revealed that the cortisol levels on Sunday were significantly lower than those on the six workdays (p < 0.05). There were no differences between males and females in cortisol levels on workdays (15.7 ± 10.48 nmol/L and 15.04 ± 5.78 nmol/L, respectively; p > 0.05) and on Sunday (11.57 ± 7.78 nmol/L and 10.75 ± 5.25 nmol/L, respectively; p > 0.05).

There was a moderate to strong correlation in the cortisol levels between workdays (Pearson’s r = 0.53-0.77, p < 0.01), except between Monday and Friday (Pearson’s r = 0.44, p < 0.05). The cortisol levels on Sundays were also correlated with those on the six workdays, however, the correlation was found to be relatively weaker (Pearson’s r = 0.30-0.65, p < 0.01) than the correlation between the cortisol levels on the six workdays.

Changes in DHEA levels from Monday through Sunday
DHEA levels were determined from the same saliva samples that were used for determining the cortisol levels (Fig. 3). The mean DHEA levels on Monday (0.93 ± 0.58 nmol/L) and Tuesday (0.90 ± 0.54 nmol/L) were lower than the other days (ranged 1.02 nmol/L to 1.18 nmol/L) (Fig. 3). The RCBD-ANOVA test revealed significant day-to-day differences in the mean DHEA levels between the days of the week (F6, 168 = 2.23, p > 0.05). Duncan’s post-hoc test revealed that the DHEA levels on Monday and Tuesday were significantly lower than those on Thursday or Friday (p < 0.05), but the DHEA levels on Saturday, Sunday, and Wednesday were not different from each other (Fig. 3). There were no differences between males and females in the overall DHEA levels on the seven consecutive days (1.0 ± 0.48 nmol/L and 1.13 ± 0.7 nmol/L, respectively; p > 0.05). The DHEA levels between workdays correlated with each other (Pearson’s r = 0.62-0.78, p < 0.01). The levels measured from Monday and Friday were weakly correlated (Pearson’s r = 0.39, p < 0.05).
DHEA levels on Sunday were weakly correlated with those on the six workdays (Pearson’s $r = 0.38-0.57$, $p < 0.05$), but the levels measured from the other workdays were well correlated with those from Monday (Pearson’s $r = 0.63$, $p < 0.01$).

**Changes in molar C/D ratios from Monday through Sunday**

The molar C/D ratios were calculated for each subject by using the data from the cortisol and DHEA levels (Fig. 4). The mean molar C/D ratios for the week were able to be divided into three subgroups, Monday and Tuesday (20.70 and 21.42, respectively), Sunday (12.12), and other workdays (range, 16.63-17.72) (Fig. 4). The RCBD-ANOVA test revealed that there were significant day-to-day differences in the mean molar C/D ratios between the days of the week ($F_{6, 168} = 8.10$, $p < 0.01$). Duncan’s post-hoc test revealed that the molar C/D ratios on Monday and Tuesday were significantly higher than those on the other four weekdays ($p < 0.05$), except Wednesday. Interestingly, the molar C/D ratios on Sunday were significantly lower than those on the other six workdays ($p < 0.05$). There were no significant differences in the molar C/D ratios among Wednesday, Thursday, Friday, and Saturday (Fig. 4). The overall molar C/D ratios on the six workdays were not different between the male and female subjects (19.81 ± 2.26 and 18.22 ± 11.31, respectively; $p > 0.05$), and the molar C/D ratios on Sunday were not different between the male and female subjects (13.82 ± 6.97 and 10.29 ± 5.78, respectively; $p > 0.05$). There was a moderate to strong correlation in the molar C/D ratios between the workdays (Pearson’s $r = 0.62-0.80$, $p < 0.01$), as well as between Sunday and the workdays (Pearson’s $r = 0.48-0.65$, $p < 0.01$).

**DISCUSSION**

The present study showed changes in the cortisol levels, the DHEA levels, and the molar cortisol to DHEA (C/D) ratios after awakening during the week in working subjects. The principal finding of the present study is that the molar C/D ratios represented well the differences in the adrenocortical status between workdays and Sunday, as well as between the first two workdays and the subsequent workdays. The cortisol levels measured 30 minutes after awakening were different between workdays and Sunday, but the cortisol levels were not different among the workdays. The molar C/D ratios 30 minutes after awakening provided additional information regarding the differences in the adrenocortical status between workdays.

To the best of our knowledge, only four papers have demonstrated differences in adrenocortical activities between workdays and the weekend, and cortisol awakening response (CAR) was a common factor analyzed in all of these studies.17-20 None of these studies analyzed the DHEA levels after the awakening period. The present study shows a clear workday-related change in the cortisol levels on workdays but not on Sunday. This result confirms our hypothesis and the results of a previous study.18 Elevated cortisol levels and more pronounced CAR on workdays is believed to reflect anticipation or preparation for a stressful work life in the approaching week. This anticipation may refer to job-related stress or the preparation of strategies to
manage upcoming tasks that may act as cognitive or internal stressors.\textsuperscript{18,20}

Since there is a tendency for the cortisol response to be attenuated after repeated identical psychological stressors,\textsuperscript{30,31} it is believed that cognitive or internal stressors on individual workdays differ from one another, as suggested by the changes in the cortisol levels. The psychological stress caused by transitioning from weekend to workweek activities is not fully explained by increased cortisol levels upon awakening on workdays or more pronounced CAR, because cortisol levels and the slope of CAR on workdays were not different from each other.\textsuperscript{19,20}

Interestingly, the molar C/D ratios 30 minutes after awakening on the first 2 workdays (Monday and Tuesday) were significantly higher than those on the other workdays or Sundays. The molar C/D ratio was affected by both the cortisol and DHEA levels; however, since the DHEA levels did not change, the increased cortisol levels observed on the first two workdays is the primary cause for the elevated molar C/D ratios. The relatively low DHEA levels on Monday and Tuesday contributed to the differences in the molar C/D ratios on the other workdays, because the cortisol levels had minimal changes on these days. These results suggest that, although the day-to-day changes in the molar C/D ratios primarily mirrored the cortisol levels, subtle changes in the adrenocortical status were amplified in measurements of the molar C/D ratios because the ratios simultaneously reflect changes in the cortisol and DHEA levels.

The molar C/D ratio has been used as an index for adrenocortical status because the ratio represents the adrenocortical steroidogenic balance.\textsuperscript{32} The relationship between cortisol and DHEA levels is considered important in understanding psychiatric, cognitive, and affective disorders. Goodyer and colleagues\textsuperscript{33} showed that persistently depressed adolescents exhibited higher C/D ratios than adolescents that have never been depressed as well as remitted adolescents. An elevated C/D ratio is associated with increased anxiety, negative mood, and deficits in various aspects of cognitive function.\textsuperscript{14} Since the degree of novelty, unpredictability, and uncertainty of a situation is considered an important factor that triggers the adrenocortical stress response,\textsuperscript{33} elevated molar C/D ratios on the first two workdays in working individuals is considered a general response to upcoming job stress or uncertainty of workload.

A population-based study demonstrated that Monday mornings and evenings were indicated to be “the worst” in terms of stress.\textsuperscript{24} Stress scores were higher on workdays than on weekends, with the score increasing on Mondays after the weekend lows.\textsuperscript{25} In the present study, 66% of the subjects choose Monday morning as the most stressful when they were asked “Which morning is the most stressful in your week?” (data not shown). Interestingly, this weekly pattern of stress coincided with the weekly molar C/D ratio rhythm. Although little information is available on the psychological and psychiatric differences between Monday and other weekdays among working individuals, it is reasonable to assume that the physical and psychological burden increases on Monday after the leisure activities of the weekend.

This study is limited by the relatively small number of participants and the narrow range of employment types. We attempted to recruit a large number of full-time working subjects, but missed samples from many participants farther decreased the number of viable subjects. Although the number of study subjects included in the present study was not as large as other studies in the same field,\textsuperscript{19,20} all the subjects included in the present work held full-time employment. None of the study subjects were unemployed, employed part-time, retired, or students. Therefore, these results are only helpful in understanding the day-to-day differences in adrenocortical activities in full-time working individuals. These results cannot be generalized to other populations. A second limitation of this study is that no systemic studies were performed to assess the impact of stress on the work environment, economics, job-related satisfaction, or work load of each participant. Further studies have been requested to define the relationship between day-to-day differences in work-related stress and day-to-day differences in adrenocortical activities.

Taken together, the present study demonstrated differences in cortisol levels 30 minutes after awakening between the workdays and Sunday. Moreover, we showed that the molar C/D ratios 30 minutes after awakening were different between the workdays and the weekend, as well as being different between the individual workdays. Although the cortisol levels and the molar C/D ratios commonly represent upcoming psychological work-related stress, cortisol levels represent the differences in the adrenocortical response between the workdays and the weekend, whereas the molar C/D ratios also represent the differences in the adrenocortical response on the first and second workday after weekend.

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