# Evaluation of the Circadian Profiles of Serum Dehydroepiandrosterone (DHEA), Cortisol, and Cortisol/DHEA Molar Ratio After a Single Oral Administration of DHEA in Elderly Subjects

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**Aging is associated with a selective decline in circulating levels of dehydroepiandrosterone (DHEA) and its sulfate, with no major changes in cortisol secretion. In young subjects, serum levels of both DHEA and cortisol are regulated according to a circadian rhythm, and an age-related attenuation of DHEA, but not cortisol, circadian rhythmicity has been reported. Several trials have evaluated the effects of DHEA supplementation in elderly subjects, although the results are still controversial. However, no data are available on the 24-hour profile of DHEA circulating levels in elderly subjects with DHEA administration. In the present study, we evaluated the circadian rhythms of DHEA, cortisol, and the cortisol/DHEA molar ratio in old subjects treated with either placebo (old-PL) or a single 50-mg dose of DHEA (old-D), both administered orally at 0700 hours. For each variable, the circadian profiles were compared with those obtained in young control subjects. The group of young subjects displayed a circadian rhythm for both DHEA and cortisol serum concentrations but no rhythm for the cortisol/DHEA molar ratio. In the old-PL group, the circadian rhythm of DHEA was completely abolished, whereas significant rhythms for both cortisol and the cortisol/DHEA molar ratio were observed. Particularly, at each time point, the cortisol/DHEA molar ratio was significantly higher in these subjects versus the young group. In the old-D group, the circadian rhythm of DHEA was completely restored and was comparable to that observed in the young group. Analogous to the observations in young subjects, the profile of the cortisol/DHEA molar ratio in old-D subjects did not display any circadian rhythmicity, the values being almost completely comparable to those observed in young controls. Our data demonstrate that the circadian rhythm of DHEA is totally abolished in elderly subjects. A single 50-mg dose of DHEA administered orally at 0700 hours restores the circadian rhythmicity of serum DHEA and almost completely normalizes the 24-hour profile of the cortisol/DHEA molar ratio in old subjects without affecting the cortisol circadian rhythm.**



IT IS WELL KNOWN that the adrenocortical secretion of the Δ5-androgens dehydroepiandrosterone (DHEA) and its sulfate declines with age.[1-6] On the contrary, no alterations[7-9] or only minor changes[10-11] in Δ4-androstenedione and cortisol secretion occur during the aging process. The biological significance of this selective age-related decline in the adrenal secretion of DHEA is still under investigation; however, a potential involvement of this phenomenon in some aging-associated pathological conditions has been suggested.[12-16] In fact, DHEA has been suggested to exert positive effects on several parameters, such as neuronal function and plasticity, immune competence, and lipid metabolism,[17-19] which appear to be impaired in aging subjects, although many actions of DHEA, such as effects on the immune system, have not yet been accepted as scientifically proven. Also, DHEA has been demonstrated to increase the bioavailability of insulin-like growth factor 1 when administered to elderly subjects,[20] and to induce bone formation in postmenopausal women.[21] All of these actions of DHEA are negatively affected by cortisol. For this reason, the above-mentioned age-related imbalance between DHEA and cortisol secretion could be considered a negative factor for human aging.

It has been demonstrated that DHEA concentrations in peripheral blood are regulated according to a circadian rhythm, and an attenuation of DHEA, but not cortisol, rhythm occurs during the aging process.[22,23]

DHEA substitution therapy in elderly subjects is still a matter of debate, and several trials have been performed with discordant results. However, in these studies, DHEA was administered according to different dosages and treatment regimens, and no data are available to answer the question of whether such treatments can replace the physiological circadian fluctuation of DHEA serum concentrations.

In the present study, we evaluated the circadian rhythm of both DHEA and cortisol serum levels and the cortisol/DHEA molar ratio in old subjects treated with either placebo (old-PL) or a single 50-mg oral dose of DHEA (old-D). For each variable, the circadian profiles were compared with those obtained in young control subjects.

## SUBJECTS AND METHODS

### *Subjects*

Nineteen old subjects and 12 young controls participated in the study. The characteristics of the subjects are reported in the Results. All subjects were healthy and free of any medication, and none used corticosteroids in the previous 6 months. No subjects used sex steroids in the past 5 years. Disorders of cognitive function were excluded by the Mini Mental State examination.[24] The Hamilton Depression Rating Scale,[25] and the Geriatric Depression Scale[26] were used to exclude the presence of depressive disorders in young and old subjects, respectively. The protocol was approved by the local Ethics Committee of the University of Parma, and the subjects provided informed consent to participate in the study.

### *Methods*

All subjects were admitted to the Clinical Center and kept on a weight-maintenance diet. On the third day, after an overnight fast, an

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intravenous catheter was placed in a vein of the forearm at 0700 hours. Blood was collected at 0800, 1200, 1600, 2000, 2200, 2400, 0200, and 0400 hours. Nighttime blood samples were obtained while avoiding awakening the subjects. Each subject was out of bed during the day and ate meals at 0800, 1200, and 1900 hours; bed time was 2130 to 2230 and 2030 to 2200 hours for young and old subjects, respectively. According to a randomized, double-blind design, old subjects received a single oral dose of DHEA (50 mg capsule, old-D group) or placebo (old-PL group) at 0700 hours, and then blood was withdrawn as previously described. No treatment was performed in the young subjects. Serum DHEA and cortisol concentrations were measured in all samples by commercially available kits (Diagnostic Systems Laboratories, Webster, TX). The sensitivity was 0.02 ng/mL and 0.3 μg/dL for DHEA and cortisol, respectively. The intraassay and interassay coefficients of variation were, respectively, 7.9% and 8.8% for DHEA and 6.1% and 8.3% for cortisol. At each sample time, the ratio of cortisol and DHEA molar concentrations was calculated.

### *Data Analysis and Statistics*

The concentration series of DHEA and cortisol and the sequences of the cortisol/DHEA molar ratio were tested for the presence of potential circadian rhythmicity by the cosinor analysis technique, which shapes the data according to the best-fitting cosine function.[27,28] Peak (single highest) and nadir (single lowest) values were identified. The characteristics of the circadian rhythm are expressed as the mesor (mean 24-hour value), amplitude (half the difference between the peak and nadir in a complete cycle), and acrophase (the clock time at which the peak occurs in a complete cycle). The sinusoidality of the cosine curves was tested according to the method of Nelson et al.[27] Statistical comparisons of the mean values between groups at the same clock time were made using the Student *t* test for unpaired data. Data are expressed as the mean ± SEM.

## RESULTS

### *Characteristics of the Subjects*

The young group consisted of 5 men and 5 women aged 31.2 ± 2.1 years with a body mass index (BMI) of 23.3 ± 1.2 kg/m$^2$. The old-PL group consisted of 9 subjects, 4 men and 5 women aged 76.4 ± 3.1 years with a BMI of 26.7 ± 1.9 kg/m$^2$. The old-D group consisted of 10 subjects, 3 men and 7 women aged 74.7 ± 2.2 years with a BMI of 27.5 ± 2.3 kg/m$^2$. Neither age nor BMI were different between old-PL and old-D groups.

### *Cosinor Evaluation of DHEA, Cortisol, and Cortisol/DHEA Molar Ratio in the Three Groups*

The circadian profiles and chronobiological parameters of serum DHEA levels in the 3 groups are shown in Fig 1 and Table 1, respectively. A circadian rhythm of serum DHEA was observed in the young group (mean cosinor $P < .01$, acrophase 0929 hours to 1327 hours). On the contrary, no significant rhythm for DHEA was detected in the old-PL group, in which a significant ($P < .001$) decrease in serum DHEA was found at each sample time in comparison to the young group. However, when the old-D group was examined, the cosinor analysis revealed the presence of a significant rhythm for the serum DHEA profile (mean cosinor $P < .002$, acrophase 0857 h to 1038 hours), with the values significantly ($P < .02$) higher at each time point versus those observed in old-PL subjects. When evaluated at 0800 hours, DHEA concentrations were higher in the old-D group versus in the young group, with a difference close to statistical significance. In the remaining sequences,

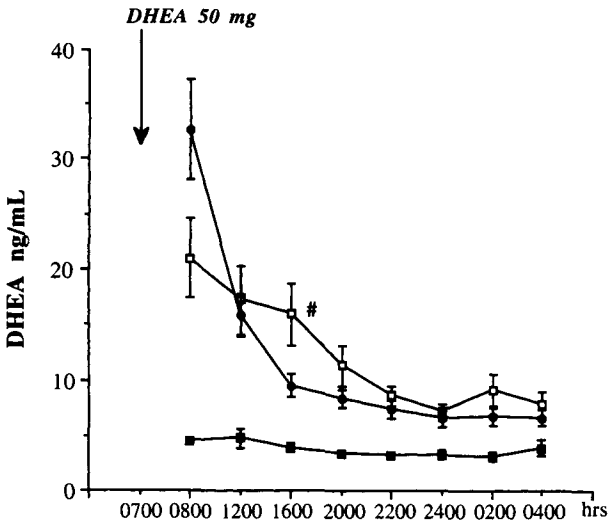


**Fig 1. Serum DHEA profiles in young (□), old-PL (■), and old-D (●) subjects. At each sample time, a significant difference was found between the old-PL and young groups ($P < .001$), as well as between the old-PL and old-D groups ($P < .02$). #$P < .05$ *v* old-D subjects in samples collected at 1600 hours.**

DHEA levels were comparable between young and old-D subjects, with the exception of higher ($P < .05$) concentrations at 1600 hours in the young group. However, both a significantly ($P < .04$) lower DHEA mesor and an earlier DHEA acrophase were observed in old-D subjects in comparison to young controls.

The circadian profiles and chronobiological parameters of serum cortisol levels in the 3 groups are shown in Fig 2 and Table 1, respectively. A circadian rhythm of serum cortisol concentrations was found in the young group (mean cosinor $P < .001$, acrophase 0809 h to 1244 hours), as well as both the old-PL group (mean cosinor $P < .001$, acrophase 0823 h to 1206 hours) and the old-D group (mean cosinor $P < .002$, acrophase 0809 h to 1321 hours). A significant ($P < .05$) difference was demonstrated for serum cortisol concentrations

**Table 1. Chronobiological Parameters of DHEA, Cortisol, and the Cortisol/DHEA Molar Ratio in Young, Old-PL, and Old-D Subjects**

| Parameter | Mean Cosinor *P* | Mesor† | Amplitude† | Acrophase Hour (95% CI) |
|---|---|---|---|---|
| DHEA | | | | |
| Young | <.01 | 13.58 ± 1.9 | 6.06 ± 1.43 | 1142 (0929-1327) |
| Old-PL | NS | | | |
| Old-D | <.002 | 3.33 ± 1.2* | 9.15 ± 1.6 | 0941 (0857-1038) |
| Cortisol | | | | |
| Young | <.001 | 10.95 ± 1.1 | 5.43 ± 0.6 | 1026 (0809-1244) |
| Old-PL | <.001 | 9.03 ± 0.9 | 3.03 ± 0.4 | 0953 (0823-1206) |
| Old-D | <.002 | 11.06 ± 0.8 | 4.26 ± 0.6 | 1022 (0809-1321) |
| Cortisol/DHEA | | | | |
| Young | NS | | | |
| Old-PL | <.04 | 19.08 ± 2.9 | 3.37 ± 0.9 | 0916 (0554-1319) |
| Old-D | NS | | | |

NOTE. DHEA is expressed as ng/mL, and cortisol is expressed as μg/dL.

*$P < .04$ *v* mesor value in DHEA young.

†Mean ± SE.

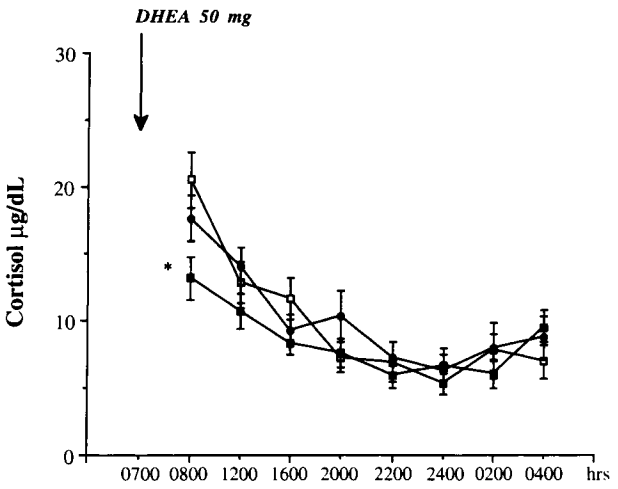


**Fig 2. Serum cortisol profiles in young (□), old-PL (■), and old-D (●) subjects. *$P < .05$ *v* young subjects in samples collected at 0800 hours.**

at 0800 hours between the young and old-PL groups, the values being lower in old subjects. In the remaining sequences, no differences between groups were demonstrated at any sample time, although a slight decrease in cortisol amplitude was found in old-PL subjects compared with both young and old-D groups.

The circadian profiles and chronobiological parameters of the cortisol/DHEA molar ratio in the 3 groups are shown in Fig. 3 and Table 1, respectively. In the young group, cosinor analysis failed to demonstrate a circadian rhythm of the values, although the results were close to statistical significance. However, in the old-PL group, the profile displayed a circadian rhythm (mean cosinor $P < .04$, acrophase 0554 h to 1319 hours), with values significantly ($P < .01$) higher at each sample time than those detected in young subjects. No significant rhythm of cortisol/DHEA sequences was found in the old-D group, in which the values were significantly ($P < .05$) lower during the entire profile than those observed in old-PL subjects. Also, a significant ($P < .03$) difference was found in values calculated at 0800 between old-D and young subjects.

## DISCUSSION

Our findings demonstrate that a single 50-mg dose of DHEA administered orally at 0700 hours restores the circadian rhythmicity of serum DHEA concentrations and almost completely normalizes the circadian profile of the cortisol/DHEA molar ratio in elderly subjects.

In agreement with previous studies,[22] our results demonstrate a circadian rhythm of circulating concentrations of DHEA in young subjects. On the contrary, in old subjects (old-PL), we found no significant rhythm of serum DHEA sequences. Studies on the age-related modifications of serum DHEA concentrations have shown that the circadian rhythm of DHEA is attenuated but still maintained in postmenopausal women.[23] In the present study, we demonstrate that the circadian rhythm of DHEA is totally abolished in elderly subjects. However, when old subjects received DHEA at a dose of 50 mg orally at 0700 hours, the profile of serum DHEA concentrations displayed a circadian rhythm, with values higher at each time point than those observed in old-PL subjects. According to our sampling time, the peak of serum DHEA concentrations in young subjects occurred at 0800 hours. For this reason, based on previous human studies from our laboratory (G. Valenti, G.P. Ceda, G. Ceresini, et al, unpublished data, April 1999) documenting a peak of serum DHEA concentrations after 1 hour from a single 50-mg oral DHEA treatment, our old subjects received DHEA at 0700 hours. With this regimen of DHEA administration, we observed a peak of serum DHEA concentrations in the old-D group which coincided with that observed in young subjects (ie, both profiles peaked at 0800 hours) with no statistical differences in peak values between the 2 groups, although the concentrations were higher in old-D subjects. However, the chronobiological evaluation of the DHEA profile showed a lower mesor and an earlier acrophase in old-D subjects compared with the young group.

A circadian rhythm of cortisol serum concentrations was found in all 3 groups of subjects. Statistical comparisons at each sample time showed no differences among the 3 cortisol profiles, documenting that DHEA administration in old subjects with our experimental conditions does not affect cortisol rhythm.

When the sequences of the cortisol/DHEA molar ratio were examined in the young group, no rhythm was found. On the contrary, a significant rhythm of the values was observed in the old-PL group, in which values calculated at each sample time were higher than those observed in young subjects. This characteristic of the cortisol/DHEA molar ratio profile observed in old-PL subjects disappeared in the old-D group in which no rhythm of the profile was found, the values being comparable in these subjects and the young group, with the exception of lower values at 0800 hours, probably due to the tendency for higher DHEA concentrations at this sample time in old-D subjects.

The age-related decline in DHEA concentrations appears concomitantly with the increase of several aging-associated

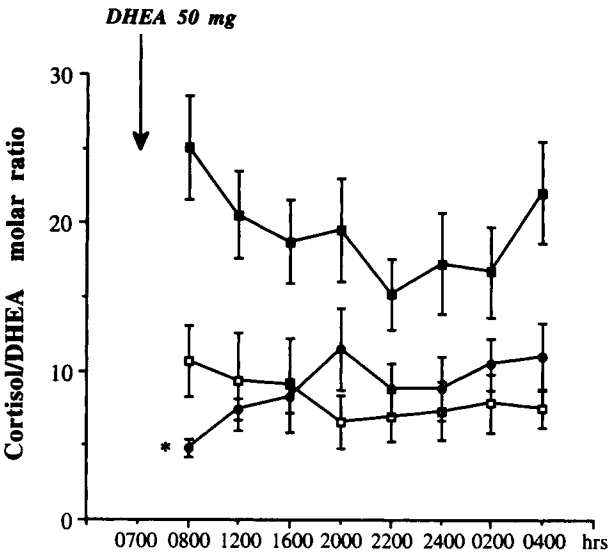


**Fig 3. Mean 24-hour profiles of the cortisol/DHEA molar ratio in young (□), old-PL (■), and old-D (●) subjects. At each sample time, a significant difference was found between the young and old-PL groups ($P < .01$), as well as between the old-D and old-PL groups ($P < .05$). *$P < .03$ *v* young subjects in samples collected at 0800 hours.**

deficits, and the increase in the cortisol/DHEA molar ratio observed in old subjects might result in negative effects on the progression of some aging-associated clinical disorders, as cortisol can exert biological effects opposite to those of DHEA on several targets, including neuronal function, immune competence, and the endocrine system. The perspective of DHEA treatment in old subjects, although still a matter of debate, has been hypothesized and several experimental trials have been performed, although the results are still controversial. However, in these studies, DHEA was administered according to different regimens. For example, in some protocols, DHEA was administered during the nighttime,[29,30] while in others it was given in the morning[31] or even 4 times per day.[32] Also, the doses were different, ranging from 50 to 1,600 mg/d. Some groups have suggested that an oral dose of 50 mg/d should be considered as a physiological DHEA replacement treatment in elderly subjects.[20] Our data are in agreement with this notion, demonstrating that oral administration of a 50-mg dose of DHEA at 0700 hours restores the circadian rhythmicity of serum DHEA concentrations in old subjects and almost completely normalizes the 24-hour profile of the cortisol/DHEA molar ratio.

These observations should be taken into account in the perspective of oral DHEA supplementation in old subjects, as well as young adult patients with adrenal insufficiency.

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