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Methodologically Critical Interactions of Circadian Rhythm, Sex, and Aging Characterize Serum Aldosterone and the Female Adrenopause[1, 2]

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Nine 20- to 26-year-old and ten 70- to 78-year-old diurnally active, nocturnally resting women in Würzburg, Federal Republic of Germany, gave blood at 0700, 1000, 1300, 1600, 1900, and 2200 for radioimmunoassay of aldosterone. Single and population-mean cosinors were applied. A multivariate analysis of circadian rhythm characteristics revealed effects of age on the amplitude ($p = .003$) but not the mesor of aldosterone in women (i.e., the age effect could only be detected at certain circadian times but not at others). No change with age was found for concomitantly sampled men. Statistically significant interactions among circadian time, age, and sex ($p = .001$) establish the adrenocorticopause in women and suggest that it may occur earlier in women than in men, in keeping with similar results on serum cortisol in the same subjects. This phenomenon awaits scrutiny from the viewpoint of its bearing on longevity and life quality. These results also indicate the importance of assessing chronobiological characteristics.

Key Words: Age, Aldosterone, Chronobiology, Human adrenocorticopause

A RECENT STUDY on adolescent, young adult, and postmenopausal North American (mostly Minnesotan) and Japanese (mostly Kyushuan) women (Halberg et al., 1981; Haus et al., 1979; Nelson et al., 1980) revealed a statistically highly significant effect of age on the circadian (about 24-hour) amplitude of plasma luteinizing hormone, estradiol, 17-OH progesterone, and dehydroepiandrosterone sulfate. A statistically significant age effect on the circadian amplitude of plasma aldosterone was also indicated but required follow-up in view of heterogeneity of variance and multiple testing, and also because an adrenopause could constitute an important aspect of both primary aging (senescence) and disease of the elderly (senility) in the sense of Busse (1979). The circannual (about 1-year) amplitude of aldosterone in human blood already has been shown to be correlated negatively with the risk of developing diseases associated with high blood pressure—stroke, and heart and kidney disease (Radke et al., 1980). Herein the age-aldosterone interaction was investigated further in representative samples of clinically healthy European men and women.

Materials and Method

Nine young women (ages 20 to 26 years), 10 old women (ages 70 to 78 years), 10 young men (ages 23 to 29 years), and 10 old men (ages 70 to 81 years) (Groups I to IV, respectively in Tables 1 and 2) on a routine of diurnal activity and nocturnal rest (2300 to 0700) volunteered with informed consent for the study in Würzburg, Federal Republic of Germany. The subjects ate at 0830, 1200, and 1830 on a given day, during which venous blood was sampled from the antecubital fossa at 0700, 1000, 1300, 1600, 1900, and 2200. Each subject contributed a sin-

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Table 1. Subjects Investigated and Serum Aldosterone: Women[a]

	Plasma (mEq/liter)		Aldosterone (ng/dl) at:					
Age (years)	Na^+	K^+	0700	1000	1300	1600	1900	2200
Group I								
20	140	4.2	11.3	9.7	10.5	11.8	1.3	0.6
21	139	3.7	31.0	24.4	31.0	9.7	8.4	8.4
22	140	3.6	8.4	11.8	11.8	12.6	17.7	17.7
23	141	3.9	24.4	29.0	15.6	11.8	9.7	12.6
23	141	4.3	10.5	8.4	11.8	6.3	3.2	5.3
24	136	3.8	24.4	14.3	11.4	7.2	9.3	10.5
24	143	3.8	8.0	6.7	6.7	15.5	6.7	3.9
25	145	3.5	20.2	8.0	8.0	21.0	4.6	2.9
26	137	3.9	9.3	9.7	15.6	13.5	8.4	6.3
Group II								
70	144	3.8	7.2	2.9	2.9	2.9	3.6	4.0
70	141	4.5	8.4	6.7	3.9	4.4	9.2	2.4
71			1.0	1.0	1.0	1.0	1.0	1.6
71	143	4.6	7.1	4.2	1.0	2.9	6.6	5.9
73	141	3.4	9.6	9.2	16.4	17.6	18.2	16.4
74	142	4.8	7.2	2.5	2.2	9.2	2.2	11.2
75			19.8	15.1	9.7	6.7	16.8	5.3
76			14.0	6.0	17.2	14.0	12.4	14.0
76	144	4.8	14.0	11.2	12.4	14.0	13.2	14.8
78			2.9	2.4	3.6	6.3	5.9	1.1

[a] 23.1 ± .6 (*SE*) and 73.4 ± .9 years of age; 167.8 ± 3.0 and 156.8 ± 1.9 cm in height; 63.3 ± 4.8 and 61.1 ± 3.5 kg in body weight, respectively. $p < .05$ at 0700, 1000, and 1300 and $p > .05$ at 1600, 1900, and 2200 in comparing groups I and II (Student's t test).

Table 2. Subjects Investigated and Serum Aldosterone: Men[a]

	Plasma (mEq/liter)		Aldosterone (ng/dl) at:					
Age (years)	Na^+	K^+	0700	1000	1300	1600	1900	2200
Group III								
23	143	3.8	9.3	10.5	13.4	24.4	3.6	24.4
25	140	4.2	17.7	31.1	42.0	16.0	27.0	28.0
25	143	4.0	12.6	7.1	11.4	6.7	13.4	2.9
25	143	3.9	8.4	14.3	8.0	12.6	12.6	18.7
26	144	4.1	20.2	11.4	12.6	14.3	5.9	3.9
26	144	3.8	14.3	6.3	5.9	4.6	2.9	4.4
26	142	4.4	11.4	35.4	12.6	10.5	4.0	1.8
29	144	4.8	40.4	35.4	13.5	23.0	16.0	6.3
29	144	3.8	15.5	13.4	9.7	9.7	9.3	4.4
29	144	4.0	24.4	9.7	16.4	9.2	6.7	8.0
Group IV								
70	141	4.5	14.8	12.8	7.6	10.0	5.2	7.6
71	140	4.4	20.8	12.4	11.2	14.8	10.0	9.2
72	140	4.4	12.0	12.0	10.0	12.2	10.4	4.4
72	142	3.9	6.0	6.0	6.0	6.0	5.2	5.8
74	140	4.9	17.2	20.0	7.6	13.2	10.0	7.6
74	139	4.0	18.0	15.6	28.0	30.4	14.8	12.0
76	136	4.8	19.2	20.8	26.4	18.0	17.2	17.2
77			14.8	11.2	14.0	9.2	10.0	8.0
79	136	3.8	37.6	48.0	46.0	60.0	60.8	34.4
81	141	3.9	19.2	14.8	22.4	8.0	10.0	8.4

[a] 26.3 ± .6 (*SE*) and 74.6 ± 1.1 years of age; 175.3 ± 2.3 and 171.1 ± 3.4 cm in height; 70.0 ± 2.6 and 75.2 ± 6.8 kg in body weight, respectively. $p > .05$ at all times in comparing groups III and IV (Student's t test).

gle set of six samples on a day between December, 1978 and May, 1979. Serum was separated by centrifugation and stored frozen at −28°C until it was radioimmunoassayed for aldosterone (lower sensitivity limit = 3.5 ± 0.5 pg; coefficients of variation were 8% for intraassay and 10.7% for interassay precision).

Data from each subject were analyzed by the single-cosinor method (Halberg et al., 1972), involving the least squares fit of a 24-hour cosine function: $f(t) = M + A\cos(\omega t + \phi)$, where ω is the angular frequency and t is time, to obtain point-and-interval estimates of the circadian mesor (M, mean value of a rhythm defined by the cosine model), amplitude (A, one-half of the difference between highest and lowest values in a rhythm defined by the model), and acrophase (ϕ, timing of the highest point in a rhythm defined by the model). Results were summarized by the population-mean-cosinor (Halberg et al., 1967) and compared by means of a multivariate analysis of rhythm characteristics using Hotelling's T^2 statistic. Mardia's (1972) test of phase differences was also applied.

Results

Mean values for plasma aldosterone in each group are plotted against time in Figure 1. Results from Student's t tests of the age difference at each circadian time available for comparison indicate an age difference in women at 0700, 1000, and 1300 but not at the three later time points. An age effect is not apparent in the results from men at any one of the six circadian test times here investigated. Table 3 shows me-

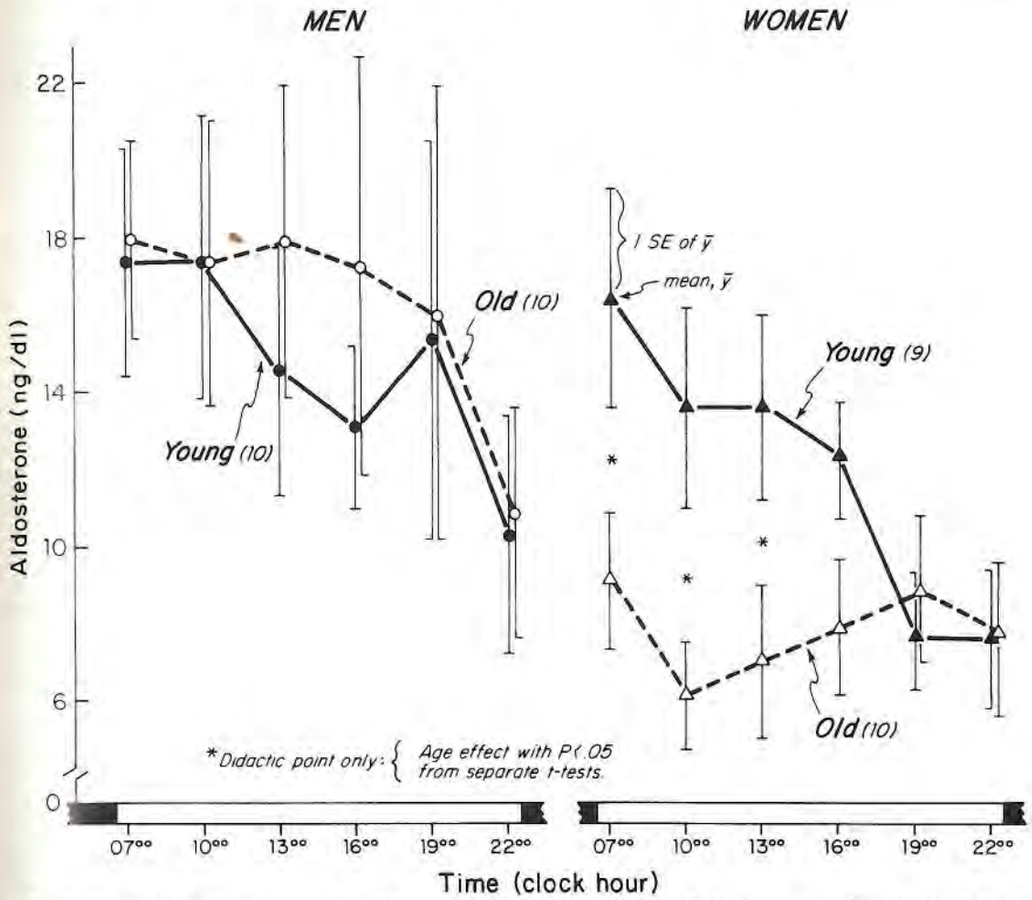

Fig. 1. Plasma aldosterone concentrations show a difference between young and elderly women only at 0700, 1000, and 1300 ($p < .001$ from analysis of variance).

Table 3. First Order Statistics: Imputations from Fit of 24-Hour Cosine Curve[a]

Sex	Age (years)	Subject no.	$M \pm SE$ (ng/100 ml)	$A \pm SE$ (ng/100 ml)	ϕ (time)
Female	20–26	1	6.4 ± 1.5	5.9 ± 2.0	1104
		2	18.8 ± 2.9	11.8 ± 3.2	0833
		3	13.5 ± 1.0	4.0 ± 1.0	2057
		4	18.2 ± 1.6	8.8 ± 1.9*	0700 (0452, 0804)
		5	7.4 ± 1.1	3.2 ± 1.2	0916
		6	14.8 ± 1.2	8.1 ± 1.8*	0507 (0336, 0636)
		7	6.8 ± 2.0	3.8 ± 3.2	1423
		8	10.3 ± 4.3	4.2 ± 5.2	1004
		9	9.0 ± 1.0	5.1 ± 1.4	1331
	70–78	10	4.6 ± .6	2.2 ± .9	0335
		11	6.0 ± 1.6	1.1 ± 2.1	0553
		12	1.2 ± .1	.3 ± .1	0222
		13	5.7 ± .8	3.4 ± 1.2	0156
		14	13.7 ± .7	5.0 ± .9*	1807 (1636, 1940)
		15	6.7 ± 2.0	3.5 ± 3.0	0038
		16	13.1 ± 2.9	4.5 ± 3.8	0555
		17	12.7 ± 2.2	1.5 ± 2.7	1830
		18	13.6 ± .5	1.4 ± .8	2352
		19	3.0 ± .9	2.4 ± 1.3	1538
Male	23–29	20	14.1 ± 4.9	3.2 ± 5.5	1946
		21	25.5 ± 5.5	5.1 ± 8.7	1338
		22	8.7 ± 2.4	1.8 ± 3.2	1113
		23	13.2 ± 1.8	3.5 ± 2.4	2317
		24	11.2 ± 2.3	5.3 ± 2.5	0901
		25	7.4 ± 1.2	4.8 ± 1.6	0534
		26	11.2 ± 4.5	11.4 ± 5.4	1006
		27	23.5 ± 4.6	12.8 ± 5.3	0728
		28	10.4 ± 1.2	3.8 ± 1.4	0824
		29	13.4 ± 2.7	6.4 ± 3.4	0633
	70–81	30	10.3 ± 1.0	3.9 ± 1.3	0634
		31	13.6 ± 1.9	3.6 ± 2.4	0632
		32	9.5 ± 1.2	3.1 ± 1.7	1144
		33	5.8 ± .1	.3 ± .2	0911
		34	13.0 ± 2.1	4.5 ± 2.5	0724
		35	16.7 ± 2.7	10.3 ± 4.4	1358
		36	18.9 ± 1.4	3.7 ± 2.1	1159
		37	11.2 ± 1.0	2.5 ± 1.1	0837
		38	43.6 ± 4.1	14.3 ± 6.5	1532
		39	13.5 ± 2.3	5.5 ± 2.6	0907

[a] M, midline-estimating statistic of rhythm described by cosine model; A, half of difference between highest and lowest value of rhythm; ϕ = time of highest value in rhythm, with 95% confidence limits. The values tabulated are considered as imputations (derived by a fit that in itself may not suffice to reject the zero-amplitude assumption) to be summarized by second order statistics (Figure 3).

* $p < .05$

sors, amplitudes, and acrophases, as the result of fitting a 24-hour cosine curve to each subject's data series. An F test of the zero-amplitude hypothesis (Halberg et al., 1972) indicates a statistically significant fit ($p < .05$) for only three subjects (i.e., the three women for whom the amplitude estimate is marked with an asterisk and confidence limits are given for the acrophase).

Figure 2 compares acrophases for aldosterone (open and closed circles) in serum of young and old women (on the left) and in that of men in two age groups (on the right). Results on serum cortisol from a separate study (Schramm et al., 1980) are included (open and closed squares). Tests of acrophase differences according to a procedure outlined by Mardia (1972) were not statistically significant in the case of cortisol, whereas a large difference in acrophase of aldosterone could be demonstrated when comparing young and old women on the one hand and old women versus old men on the other hand

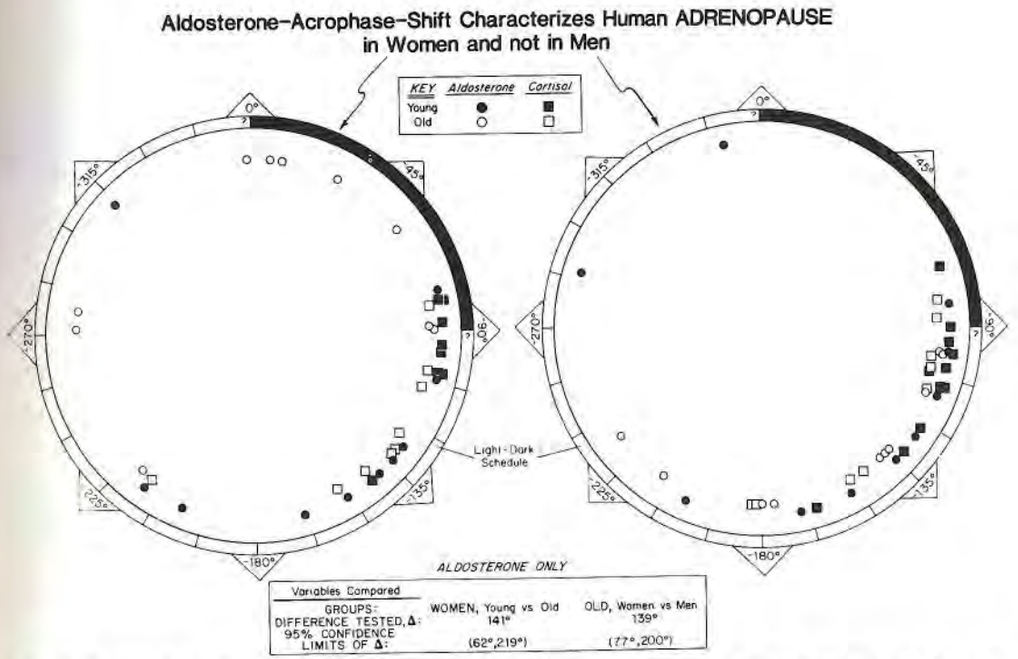

Fig. 2. Timing of circadian aldosterone rhythm differs with statistical significance between young and elderly women and between elderly women and men of corresponding age.

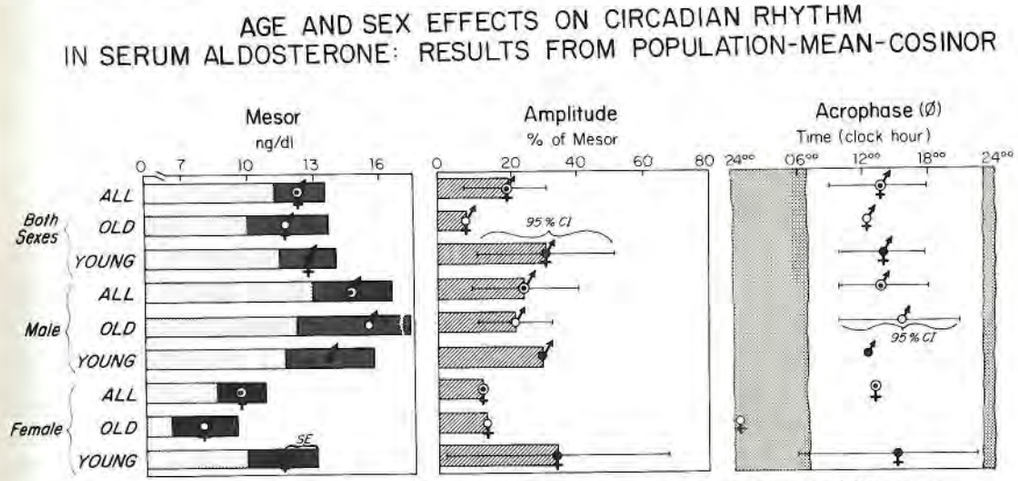

Fig. 3. Comparison of circadian parameters for serum aldosterone between women and men of different ages.

($p < .05$).

The results provided by the single cosinor method can be considered as first order statistics—as so-called imputations—for further summary by second order statistics, namely by a multivariate analysis of rhythm characteristics and by the population mean-cosinor method (Halberg et al., 1967). These analyses were performed with individual amplitude imputations expressed in (a) absolute units (ng/dl) and (b) as a percentage of the corresponding individual mesor (see Figure 3). Estimates of rhythm char-

Table 4. Sex-Related Effect of Aging in Circadian Parameters of Serum Aldosterone[a]

Sex	Age	n	Rhythm characteristics				
			M (ng/100 ml)	A (ng/100 ml)	ϕ (degrees)	Mean A	A:M
Female	Young	9	11.72 ± 1.59	3.52 ± 1.46	−141 ± 22	6.09 ± 0.97	58.50 ± 5.97
	Old	10	8.02 ± 1.51	.86 ± .50	−8 ± 52	2.52 ± 0.49	37.50 ± 7.22
Male	Young	10	13.83 ± 1.89	3.75 ± 1.58	−127 ± 15	5.81 ± 1.13	44.34 ± 8.10
	Old	10	15.59 ± 3.32	3.29 ± .96	−178 ± 28	5.15 ± 1.30	31.29 ± 4.75

[a] n = number of subjects; rhythm characteristics are given as group mean ± 1 SE; ϕ: 360° = 24 hours; 0° = local midnight.

Table 5. Results from Tests of Differences in Rhythm Characteristics by Multivariate Analysis as Functions of Age and Sex

Comparison	Age effects				Sex effects			
	Females		Males		Young		Old	
	F	p	F	p	F	p	F	p
(M, A, ϕ)	4.0	.03	.7	.57	.3	.86	9.9	.001
(A, ϕ)	5.1	.02	1.0	.40	.1	.88	14.1	<.001
M	2.8	.11	.2	.65	.7	.41	4.3	.05
A	11.5	.003	.1	.71	<.1	.86	3.6	.07
A:M (%)	2.8	.11	1.9	.18	.8	.38	.5	.48

acteristics for each of the four groups studied and for various pools of data from the same sex or age are shown in Figure 3 and Table 4. Acrophases seem to be similar, except for the group of old women. The mesors appear to be smaller in women than in men. The amplitudes also seem to be smaller in the groups of elderly men or women as compared with the corresponding group of younger subjects.

The 24-hour cosine function accounted for 69 ± 9% and 48 ± 9% of total variability in data from young and old women, respectively (percentage of rhythm for pool = 58 ± 7). In the case of young and old men the fitted model accounted for 47 ± 9% and 58 ± 4% of the corresponding total data variability (percentage of rhythm in pool = 53 ± 5). When one pools all data from young subjects irrespective of sex, the percentage of rhythm is 58 ± 6, whereas the corresponding result on the pool from all old subjects is 54 ± 5. The overall pool of all data, irrespective of sex and age, yields a percentage of rhythm of 55 ± 4%. The data are limited, however, and do not suffice for a consistent demonstration of a rhythm by the population-mean cosinor method. Actually, a population rhythm cannot be demonstrated in the data from women except for the young group after transformation into percentage of mesor, in which case statistical significance reaches the 5% level. In the case of the data on men the p values for the population-mean cosinor are consistently favorable, notably for the old men and for the pool of data from young and old men.

As shown by a multivariate analysis (Table 5) the clearest effect of aging is a sex-related decrease in circadian amplitude of aldosterone, a statistically significant difference ($p = 0.003$) is found only between young and senescent women.

Discussion

Much effort has been expended to investigate the adrenocortical glucocorticoid-producing system during aging, including studies of circadian rhythms, yet little is known about age-associated changes in rhythms of the main mineralocorticoid in man, aldosterone. A decrease in old subjects of several conventional indices has been reported. Flood et al. (1967) found a reduction in secretion rate, metabolic clearance rate, splanchnic extraction, and indirectly assessed plasma aldosterone. A decrease in urinary and plasma aldosterone has been documented directly by others (Crane & Harris, 1976; Kowarski et al., 1974; Sambhi et al., 1973). The previously mentioned studies concerning the secretion and metabolism of aldosterone were performed on male subjects, whereas the results on plasma aldosterone were undefined according to sex. The urinary 24-hour excretion rate was seen to be decreased in the aged of both sexes, although the decrease appeared to be greater in women. Another reported age-related modification is a decrease in aldosterone responsiveness to a variety of physiological stimuli such as upright posture and sodium-volume depletion (Crane & Harris, 1976; Weidmann et al., 1975). This is the status quo for considering the present results.

The major effect of age on the circadian rhythm of serum aldosterone here noted is a decrease in the extent of fluctuation, namely the amplitude, a finding that extends the scope of results on Minnesotan and Kyushuan women (Halberg et al., 1981; Haus et al., 1979; Nelson et al., 1980). Moreover, our results herein indicate that the age-related change in circadian aldosterone amplitude is detectable (at the ages tested) in women in the Federal Republic of Germany.

When changes in the circadian adrenal cycle in the later decades of life are discussed, it must be kept in mind that plasma renin activity declines with age (Crane & Harris, 1976; Sambhi et al., 1973; Weidmann et al., 1975). In a chronobiological investigation in our laboratory on the circadian rhythm in plasma renin activity of 10 young and 13 old subjects studied in recumbency from 8 to 32 hours, a decrease in mesor ($p < .001$) and in amplitude ($p < .05$) and possibly a delay of acrophase have been found in the older group.

The age-related decrease in the mesor of urinary excretion of norepinephrine and epinephrine, accompanied by a decrease in circadian amplitude (Descovich et al., 1974), can be viewed in the light of a reported rise in urinary dopamine (Horky et al., 1975), suggesting an impairment in dopamine β-oxidase activity in the aged. An altered norepinephrine:dopamine ratio could be of importance since the catecholaminergic nervous system has been viewed as interacting with renin, aldosterone, and cortisol rhythms (Cugini et al., 1977). A dopamine excess per se is of interest because of the dopaminergic inhibitory influence on both glucocorticoids (Werder et al., 1970) and mineralocorticoids (Sowers et al., 1980).

The decrease in the circadian amplitude of aldosterone in postmenopausal women, but not in old men, may perhaps be explained as follows: Estrogenic compounds are known to interfere with the steroid-binding proteins (Mills, 1962); serum-binding proteins facilitate the transport of steroids across the plasma membrane into the target cell (Burton & Westphal, 1972) and modify the hepatic extraction (Keller et al., 1969). The decrease in extent of plasma aldosterone fluctuation, thus, may be due merely to impairment in splanchnic extraction or blood flow related to the failure of ovarian function. The lack of estrogens may also yield an alternative explanation that refers more directly to the circadian function of the adrenal cortex. In this respect it must be pointed out that estrogens can elicit increases in the rates of aldosterone excretion, in plasma renin-substrate concentration, and in renin activity (Crane & Harris, 1962; Katz & Beck, 1974). On a theoretical basis, the lack of an adequate supply of estrogens in older women could lead to a lesser stimulation of the zona glomerulosa by renin-angiotensin. Chronobiological as well as homeostatic studies on plasma renin and renin-substrate (Crane & Harris, 1976) did not detect any sex differences thus far in potassium metabolism at different ages. It is postulated that the absence of adequate estrogenic activity or, more precisely, the menopause-related decrease in mesor and amplitude of estrogen rhythms could result in a lower circadian adrenocortical responsiveness to stimuli, possibly related to a decreased sensitivity of aldosterone adrenoreceptors. This supposition awaits experimental scrutiny; it is corroborated, however, by the observation made by our research group that a similarly sex-dependent phenomenon characterizes the cortisol circadian amplitude (Schramm et al., 1980), supporting a broader relationship between menopause and adrenopause (Table 6). A decrease of circadian amplitude for the main adrenocortical hormones, aldosterone and cortisol, thus, could be considered a sign of an age-dependent decline of adrenocortical function. Accordingly, the women's adrenopause appears to precede that of men, a circumstance with possible adjustment value in modern society.

For an integration of these findings with performance, the interested reader can be referred to a report on circadian changes in residents of a senior citizens' home, including measurements of eye-hand coordination, grip strength, and cardiovascular variables (Scheving et al., 1974).

Definition of Terms Used

Circadian: about 24 hours
Circannual: about 1 year
Mesor, *M*: mean value of a rhythm defined by a mathematical model (i.e., cosine curve)

Table 6. Age-Related Changes in Characteristics of Circadian Hormonal Rhythms in Clinically Healthy Women and Men[a]

Rhythm characteristic(s)	Sex	Hormone: Somatotropic hormone	Prolactin	Follicle-stimulating hormone	Luteinizing hormone	Cortisol	Aldosterone	Free thyroxine	Insulin	C-peptide	C-peptide
M	F			I***	I**	D*		D*		I**	I**
	M			I***						I**	I*
A	F		D**			D**	D**				I**
	M		D*	I**						D**	
(*A*,ϕ) or (*M*,*A*,ϕ)	F	Δ**			Δ***	Δ**	Δ*				
	M		Δ*						Δ*	Δ**	

[a] Results from Hotelling T^2 on circadian characteristics imputed from radioimmunoassayed blood (drawn at 0700, 1000, 1300, 1600, 1900 and 2200) from 10 men (M) and nine women (F) in each of two groups, one 20 to 29, the other 70 to 81 years of age. I, increase; D, decrease; Δ, change.

* $p \leq .05$
** $p \leq .01$
*** $p \leq .001$

Amplitude, *A*: one-half of difference between highest and lowest value in a rhythm defined by a mathematical model (i.e., cosine) applied to all available data (rather than by possibly fortuitous extreme values)

Acrophase, ϕ: timing of the highest point in a rhythm defined by a mathematical model (i.e., cosine) applied to all available data (rather than by possibly fortuitous extreme value)

Single-cosinor method: estimates rhythm parameters (M,A,ϕ) by least squares fit of cosine curve to a single time series of data

Population mean-cosinor method: estimates population rhythm parameters on the basis of respective parameter estimates obtained from subjects by single-cosinor method

Rhythm: periodic component of biological time series demonstrated by inferential statistical means

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