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Circadian timing of serum cortisol in patients with anorexia nervosa / *Timing del ritmo circadiano del cortisolo sierico in pazienti con anoressia nervosa*

Boguslawa Baranowska *, Maria Lazicka-Frelek *, Barbara Migdalska *, Stefan Zgliczynski*, Barnett Zumoff, R.S. Rosenfeld**, Germaine Cornelissen***, Helmut Arbogast****, Elke Eckert***, Franz Halberg*****

* Endocrinology Clinic, Medical Center of Postgraduate Education, Bielanski Hospital - Ceglowska 80 - 01-809 Warsaw, Poland
** Department of Medicine, Beth Israel Medical Center, New York, NY, USA
*** University of Minnesota, Minneapolis, MN, USA
**** Kinderzentrum, University of Munich, FRG

Introduction

The hypothalamic-pituitary-adrenal network in patients with anorexia nervosa (AN) appears to be unusually active. Time-unspecified fasting or other serum cortisol concentrations are reported as elevated in virtually all studies investigating plasma cortisol in patients with AN (3, 6, 8, 10, 11, 19, 36, 37). Elevated serum cortisol has been regarded as a differential diagnostic feature distinguishing AN from Addison's disease. This elevated serum cortisol has been associated with a decrease in the rate of cortisol metabolism in the form of an increased, if circadian stage-unspecified cortisol half-life (3, 8), probably related to a reduced capacity of the liver for ring A reduction of cortisol.

Elevated serum cortisol has also been associated with an increased

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rate of cortisol production relative to body size (35). There is an increase in the excretion of unconjugated or « free » cortisol in the urine, usually a very sensitive indicator of adrenal overactivity (3, 35). Adrenocortical activity is not as readily suppressed in patients with AN (8, 12) as in clinical health (CH), a finding suggesting that the hypothalamic and pituitary centers coordinating adrenal activity have an altered sensitivity.

It has been suggested (7, 29) that malnutrition in anorexia nervosa patients is responsible for alterations seen in cortisol metabolism. A more recent review (35), however, has concluded that additional changes in AN are not accounted for by protein-calorie malnutrition (29).

There are differing reports concerning the circadian variation of circulating cortisol in AN. Some authors report an absence of the usual circadian variation (10, 11, 18, 33, 36), suggesting a disturbance of the hypothalamic coordination of pituitary-adrenal function in patients with AN. Other authors report the usual circadian variation of cortisol (3, 6, 8). Studies reporting usual (non-deviant) and deviant results in circadian variation of serum cortisol are listed in Table 1.

Materials and methods

We studied 22 women with AN, ranging in age from 17 to 29 years (mean: 23 years) with weight deficiency ranging from 24% to 57% (mean 39%), and 18 CH women, 19 to 58 years of age (mean: 35 years) whose body weights were within accepted limits. AN was diagnosed based on criteria described elsewhere (9, 25):

1) a pattern of behavior aimed at inducing weight loss,

2) emaciation to a body weight at least 20% below standard,

3) cessation of menstruation for at least 3 months,

4) absence of other (overt) physical or psychiatric illness.

Ideal body weight was calculated in kg from the formula: height in cm - 100. The clinical data are presented in Table 2.

The subjects with AN or in CH were examined on the 4th day of their hospitalization. The subjects slept or rested from about 23^{oo} until 07^{oo}. Meals were served at 08^{oo}, 13^{oo} and 17^{oo}. No drugs were administered. Activity was limited to that compatible with standard hos-

Table 1

CIRCADIAN VARIATION OF SERUM CORTISOL IN ANOREXIA NERVOSA

Authors Studies reporting	Sampling deviant results	#Samples /24 h	# of subjects	Age (yrs)	% loss in body weight	Comment (% deviant)
Frankel and Jenkins (10)	09^{00}, 00^{00}	2	4	16-28	23.3-34.6 kgx	50
	08^{30}, 16^{00}	2	9	13-25	26-48xx	Data regarded as insufficient by authors
Garfinkel et al. (11)	Morning & afternoon	2	101	11-61	0-59	53
Hurd et al. (18)					33.6±10.5xx	55
Vigersky et al. (33)	08^{00}, 17^{00}	2	11	23.7±8.1	15-54	48% deviant & 22% with « reversal » of circadian rhythm
Warren & Van de Wiele (36)	09^{00}, 16^{00}	2	23	10-23		
					Studies reporting non-deviant results	
Boyar et al. (3)	q 20 min	72	10	16-28	23.8 — 40.1xx	0
Casper et al. (6)	08^{30}, 20^{00}	2	20	14-32	16 — 48xxx	0
Doerr et al. (8)	q 30 min	48	16	13-29	23 — 49xx	0

x% loss not reported
xx% loss from ideal body weight taken from the Metropolitan Life Insurance Co. Statistical Bullettin, 1959
xxx% loss from « normal »

Table 2

CLINICAL DATA OF PATIENTS WITH ANOREXIA NERVOSA (AN)

Case number	Age (years)	Height (cm)	Body weight (kg)	Deficit of body weight * (%)
1	27	156	24	57
2	26	160	28	53
3	25	165	31	52
4	29	161	31	49
5	25	162	33	47
6	22	167	37	45
7	25	168	38	44
8	22	158	34	41
9	19	163	37	41
10	26	166	39	41
11	22	172	44	39
12	28	154	34	37
13	24	160	39	36
14	19	162	40	35
15	16	165	43	34
16	18	154	38	29
17	18	155	40	27
18	17	164	48	25
19	20	160	45	25
20	26	154	41	24
21	22	161	47	23
22	27	164	49	23

* in relation to ideal body weight in kg computed as height in cm - 100

pital conditions. The patients did not exhibit any body weight gain prior to blood sampling for cortisol determinations. Blood samples were taken from the antecubital vein at 08^{00}, 12^{00}, 16^{00} and 22^{00}. Serum cortisol concentrations were measured by a fluorometric method (30). The data were analyzed by the single cosinor procedure (15, 17). Time-specified reference intervals, notably prediction intervals, were computed, as noted elsewhere (13, 21, 22), from the data on CH women and also from a data base used for international reference standards (14). Calculations were made by computer (Mera 400 and PDP11/34) with Fortran IV programs.

Results

The mean 24-h cortisol concentrations in the CH women ranged from 6.10 to 25.55 μg/dl (Table 3). The mean 24-h cortisol concentrations of the AN subjects were higher, from 12.12 to 43.28 μg/dl (Table 4). The difference between the mean of serum cortisol in AN patients of 20.9 as compared to 13.2 in CH subjects is 7.8 ± 2.1 (SE), the corresponding t is 3.65 ($p < 0.01$).

A group rhythm is demonstrated by the population-mean cosinor technique for AN and CH, as shown in Table 5. The P-values (<.01) in testing the zero-amplitude (no rhythm) assumption demonstrate the prominence of circadian rhythmicity.

The timing of the cortisol rhythm in each individual is shown in Figure 1 by acrophases alone. These are plotted along a circular scale, irrespective of amplitude. The acrophases of the CH women (dots) ranged from 06^{24} to 10^{41} (hrs min) from local midnight. Acrophases similar to those of the healthy group are also found in 16 of the women with AN (triangles). The acrophases of the six remaining patients with AN lie far outside the range of CH women (15° = 1 h). In these women (with outlying acrophases), the body weight deficit ranged from 39% to 57% of ideal body weight. The weight deficits of those AN patients with acrophases within or outside the CH limits averaged 33.4% and 48.8%, respectively. The difference of $15.4 \pm 3.8\%$ (SE) is associated with a t of 4.06 and is significant below the 1% level. One may also use a reference prediction region (ellipse) or conservative prediction limits (i.e., the tangents drawn to the ellipse) instead of a range for interpreting the acrophase. The institutional reference ellipse and the individual (A, ∅) pairs for AN patients are shown in Figs. 2a and 2b. In Fig. 2a, the amplitude is used in original units, whereas in Fig. 2b, it is expressed as percent of MESOR. With either approach, the results are discriminating in that the difference in body weight loss of patients whose cortisol (A, ∅) pair lies inside or outside the reference prediction ellipse (or the prediction limits for ∅) is statistically significant below the 1% level.

Both approaches are given (for completeness), but a priori, it was decided to interpret results on the basis of the amplitude as percentage of the MESOR, since, earlier, such reference regions, as compared to those based on the amplitude expressed in original units, had been found to be more reliable and internationally valid (21). Figs. 2a and 2b allow

Table 3

RHYTHMOMETRIC SUMMARY OF SERUM CORTISOL IN CLINICALLY HEALTHY WOMEN *

Subject ID	(μg/dl) MESOR	Amplitude	Acrophase 360° ≡ 24 h 0° = midnight degrees	hour	minute
1	10.52	2.08	— 132	08	51
2	6.10	3.14	— 142	09	30
3	14.25	5.70	— 120	08	02
4	10.98	6.98	— 102	06	50
5	10.70	3.33	— 110	07	22
6	11.23	5.74	— 131	08	47
7	10.80	2.94	— 149	09	57
8	12.33	3.16	— 129	08	39
9	10.72	3.41	— 133	08	52
10	13.80	2.69	— 106	07	07
11	11.78	3.57	— 122	08	11
12	7.55	2.35	— 96	06	24
13	11.23	4.70	— 144	09	38
14	25.55	7.15	— 124	08	16
15	22.75	6.67	— 109	07	17
16	22.50	3.98	— 148	09	52
17	10.78	4.65	— 118	07	55
18	13.22	4.28	— 160	10	41

* Each subject contributed 4 samples at 06^{00}, 12^{00}; 16^{00} and 22^{00}.

an examination of the AN subjects ranked in order of decreasing severity of body weight loss. Most of the AN patients with the largest deficit (with the lowest numbers shown in Figs. 2a and 2b) are outside the 90% prediction region.

A Kruskal-Wallis test was performed to compare the body weight loss in AN patients (in Warsaw) having their cortisol (A, $\varnothing$) pair inside or outside the reference region. A statistically significant difference is thus demonstrated ($X^2_{(1)} = 9.136$; $P < .01$). This result is in keeping with that of the Student t-test reported above. A linear regression of only borderline statistical significance ($P = .065$) was found between relative body weight loss and the extent of departure of the cortisol rhythm from « normalcy » (d_i), as gauged by the euclidean distance between the patients' (A_i/M_i, $\varnothing_i$)s and the CH population rhythm estimate (A/M, $\varnothing$):

$$d_i = [(\beta_i - \beta)^2 + (\gamma_i - \gamma)^2]^{1/2}$$

$$\text{with } \beta = \frac{A}{M} \cos \varnothing \text{ and } \gamma = -\frac{A}{M} \sin \varnothing.$$

Table 4

RHYTHMOMETRIC SUMMARY OF SERUM CORTISOL IN WOMEN WITH ANOREXIA NERVOSA *

Case #	(μg/dl) MESOR	Amplitude	Acrophase 360°≡24 h, 0° = midnight degrees	hour	minute
1	18.88	3.70	— 17	01	08
2	12.32	3.61	—209	13	58
3	16.28	0.86	— 9	00	38
4	23.28	2.57	— 94	06	16
5	28.07	10.48	—244	16	18
6	25.72	7.98	—183	12	13
7	15.33	4.58	—113	07	35
8	24.63	4.26	—113	07	32
9	23.33	6.14	—139	09	16
10	19.52	3.96	— 97	06	30
11	17.33	6.25	—261	17	25
12	15.03	4.42	—121	08	04
13	17.22	5.22	—125	08	20
14	43.28	3.74	— 95	06	21
15	15.40	5.29	—127	08	28
16	15.22	1.68	— 93	06	13
17	24.70	1.64	— 99	06	37
18	24.33	4.26	—113	07	32
19	36.07	12.73	—105	07	03
20	12.12	0.87	—145	09	42
21	16.67	1.87	—112	07	28
22	15.35	2.17	—106	07	07

* Each subject contributed 4 samples at 06^{00}, 12^{00}, 16^{00} and 22^{00}.

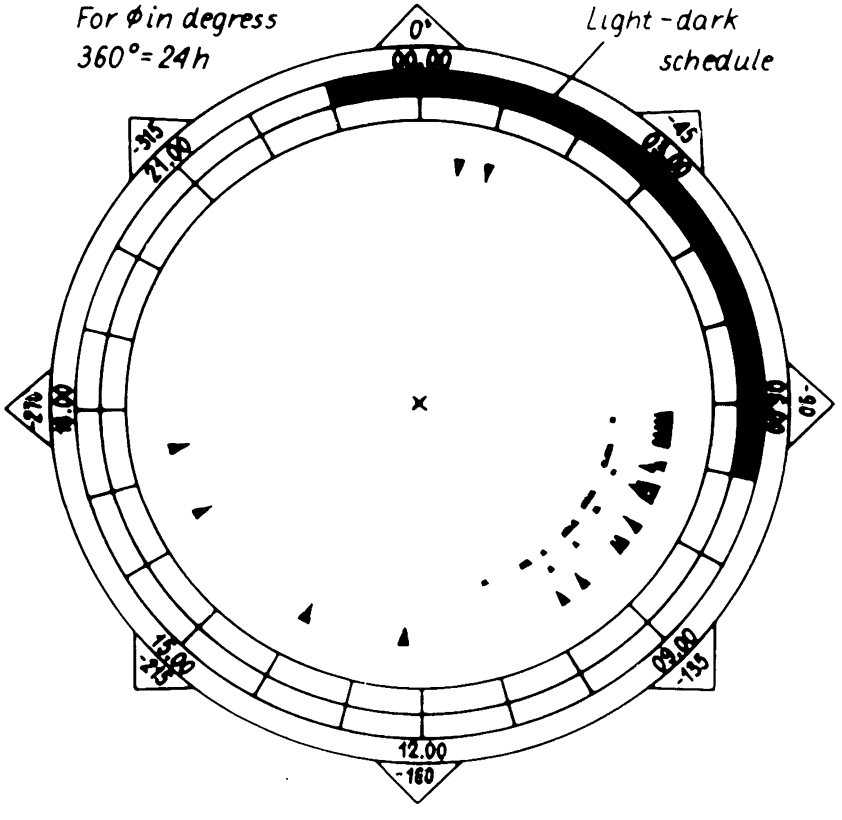
Table 5

POPULATION-MEAN COSINOR OF 24-h PROFILE OF SERUM CORTISOL CONCENTRATION IN CLINICAL HEALTH AND ANOREXIA NERVOSA *

Population	N subj.	P	M±SE	A (95% CL)	Øhr^{min} (95%CL)
Anorexia nervosa	22	.003	20.93±1.70	2.55 (1.04, 4.06)	08^{48} (07^{00}, 11^{52})
Clinically healthy women	18	<.001	13.16±1.23	4.07 (3.29, 4.86)	08^{23} (07^{48}, 09^{00})

* N subj. = number of subjects
M = MESOR
A = amplitude
Ø = acrophase
95% CL = confidence limits
P = P-value in testing zero-amplitude (no-rhythm) hypothesis

Figure 1



Acrophases from cosinor summaries of 24-h profiles of serum cortisol concentration in clinical health (●) and anorexia nervosa (▲).

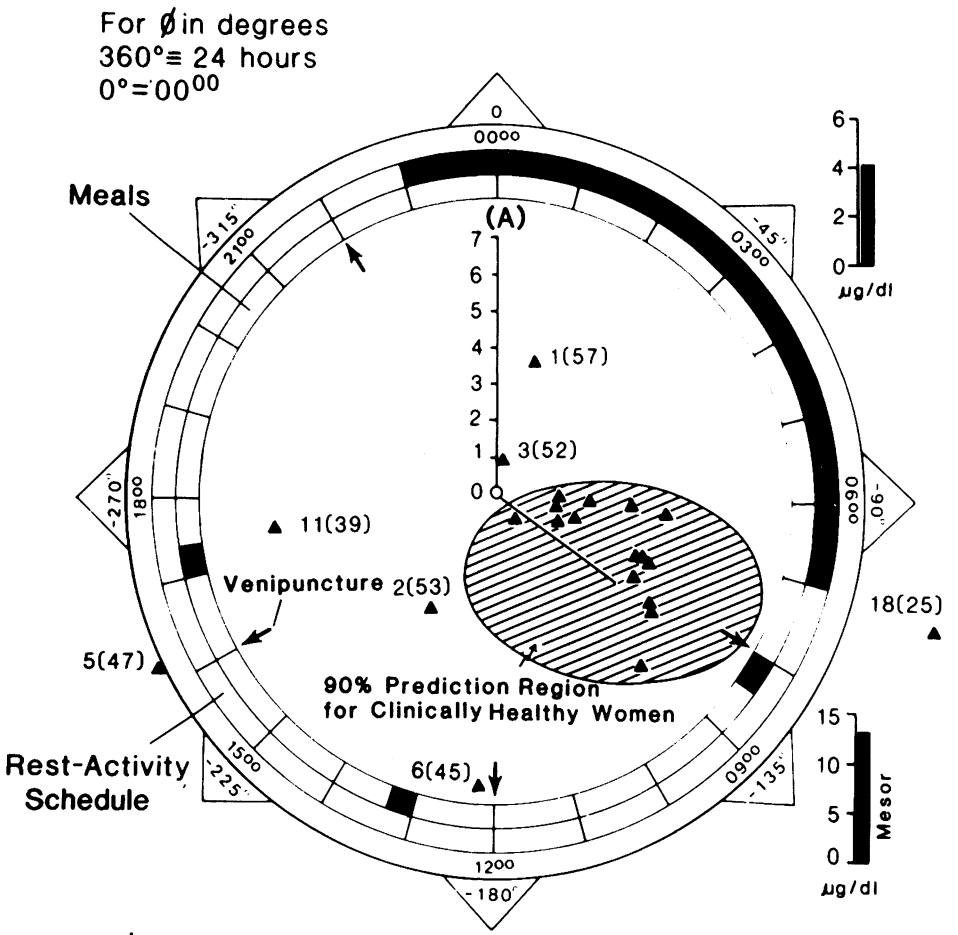
Until more data are available, one cannot say whether there may not be a gradual departure from « normalcy » as a function of body weight loss or, rather, that abnormality occurs more abruptly once body weight loss has reached a critical point.

With 4 samples, 4 to 8 hours apart, on 18 to 22 subjects, it is tempting to ignore individual behavior altogether and to restrict one's attention to groups only, unless a reference group of more than 18 clinically healthy subjects (sampled sparsely) can be found as an additional reference standard. Additional data for use as standards for women

Figure 2a

Circadian Amplitude (A)– Acrophase (Ø) Pair of Serum Cortisol in Women with Anorexia Nervosa (▲) in Relation to 90% Prediction Region for Clinically Healthy Women*

Computed with A in original units.

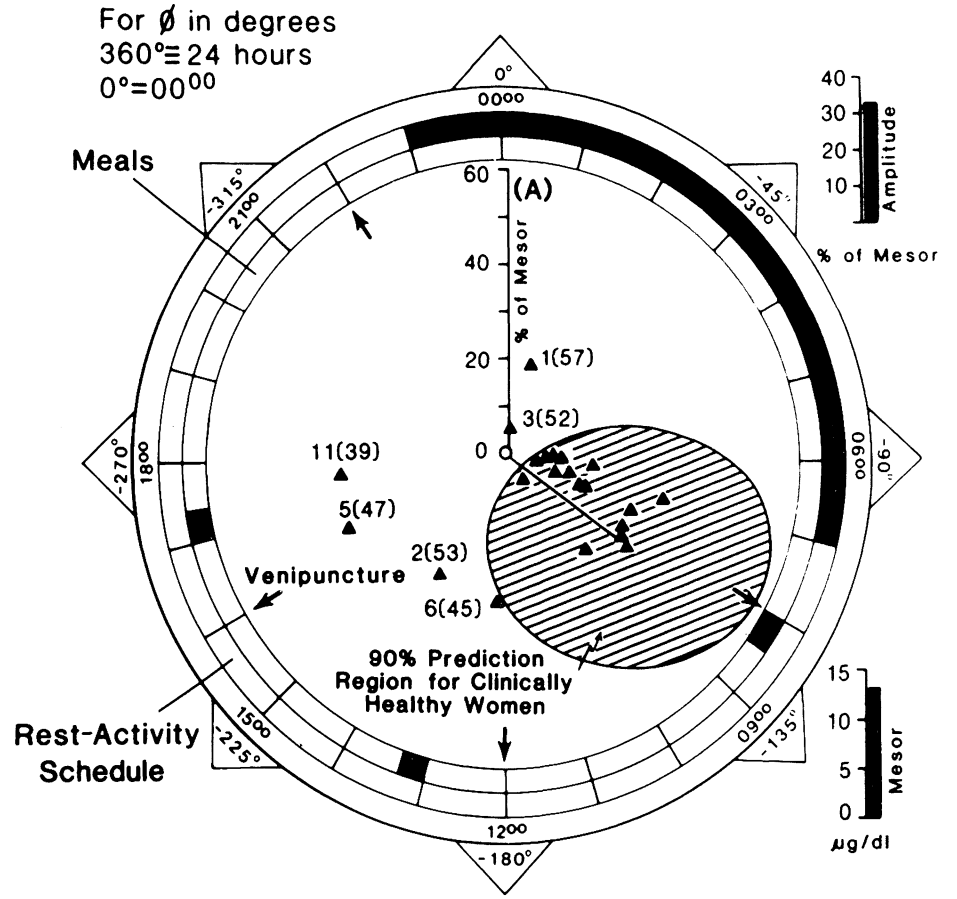


* Numbers accompanying outliers indicate rank in relation to decreasing severity of body weight loss, the loss itself being given in parentheses as percentage of ideal body weight.

Institutional elliptical reference region for acrophase-amplitude pair of serum cortisol concentrations estimated for clinically healthy women against which individual values for patients with anorexia nervosa are shown. Numbers show ranks in order of decreasing severity of body weight deficit. Deficits also given in parentheses as percentage of ideal body weight. Reference region based on amplitude in original units (a), left, and on amplitude expressed in percentage of mesor (b), middle. Results shown against international reference standard (c), right.

Circadian Amplitude (A)- Acrophase (Ø) Pair of Serum Cortisol in Women with Anorexia Nervosa (▲) in Relation to 90% Prediction Region for Clinically Healthy Women*

Computed with A as percentage of mesor.



*Numbers accompanying outliers indicate rank in relation to decreasing severity of body weight loss, the loss itself being given in parentheses as percentage of ideal body weight.

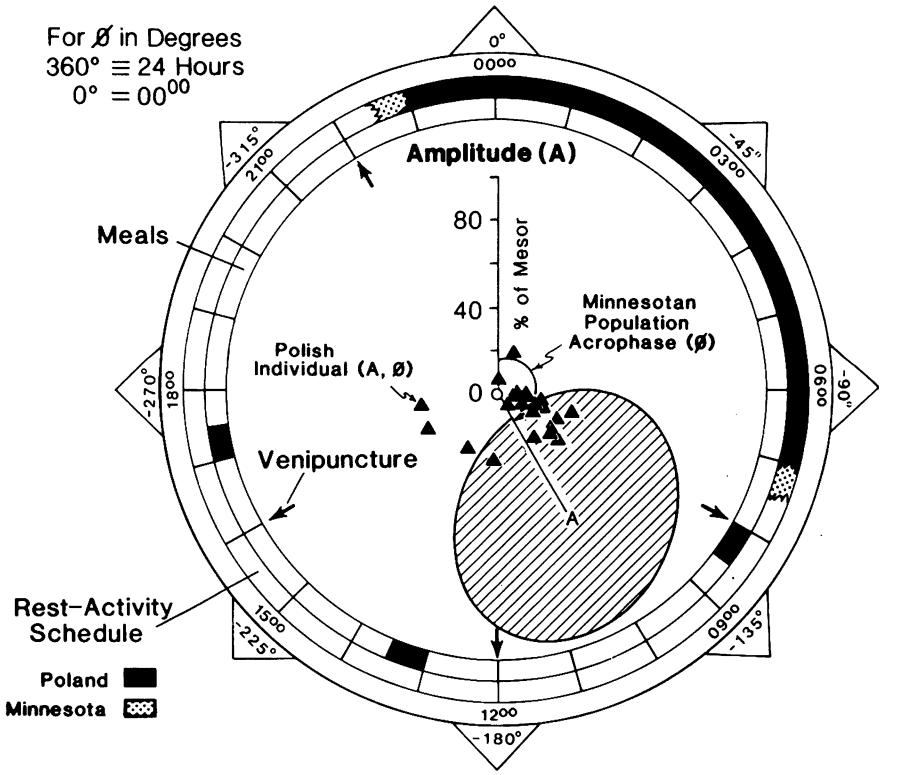
in CH are indeed available from a study involving 134 series sampled at 20-minute intervals for 24 hours, Figure 3 (14). International validity has been documented for such a reference standard in the form of the 90% prediction region for the amplitude and acrophase pair, when the amplitude is expressed as a percentage of the MESOR. Such a Minnesotan prediction region, computed to contain 90% of a population, contains the (anticipated) majority of amplitude-acrophase pairs from studies of clinically healthy individuals in Japan (23), Italy and Germany (13). Moreover, all 18 of the Polish women in CH fall within this international reference standard (not shown). By contrast, one-half of the Polish women with AN fall outside this international standard (Fig. 2c). Until proof is offered to the contrary, a local institutional reference region is preferred to an international one. In our case, the results obtained with the use of an international standard support the inferences based upon the use of a local standard.

Whichever standard is used, certain AN patients, but not all of them, have atypical acrophase-amplitude pairs. It is of interest to see whether such differences are due to amplitude only, to acrophase only, or to both characteristics. Student's t-tests for any difference in body weight loss between those with amplitudes inside and outside the (A, $\varnothing$) prediction region or inside and outside the reference interval for A, computed on the basis of the amplitude in original units, or on the basis of the amplitude expressed as percent of MESOR, were all associated with a P above the 10% level (the corresponding t-values are 0.98, 0.43, 1.39 and 0.99).

A procedure similar to that practiced for examining discrimination of extent of body weight loss by the use of acrophases as criterion was also applied to the mean 24-h cortisol concentrations or MESORS. Four patients with AN had their rhythm-adjusted means outside the range of those of CH women by more than 1 μg/dl. These four subjects had a mean weight deficit of 38%, whereas the other AN patients, who had a cortisol MESOR within the range of MESORS for CH, had a mean weight deficit of 37.5% ($t = 0.08$; $P > 0.50$). Until proof is offered to the contrary, the increase in mean cortisol concentration seen in AN patients may be non-specific in reflecting a low body weight, while a change in acrophase is more specific in this context. It is concluded, in the light of such results, that the best criterion among the three parameters examined here--MESOR, amplitude and acrophase--is indeed the acrophase. The value of a combination of these indices and of the waveform (when denser data allow its assessment, e.g., by harmonics), however, remains

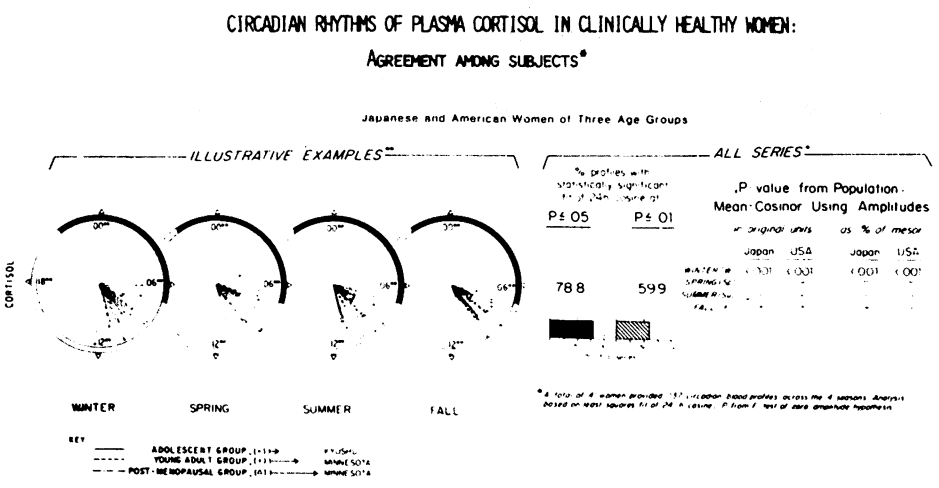
Figure 2c

Circadian Characteristics of Serum Cortisol in 11 of 22 Women with Anorexia Nervosa in Poland Lie Outside 90% Prediction Region for Clinically Healthy Minnesotans



to be investigated. For the time being, the finding of deviant cortisol acrophases relative to body weight loss is based on sparse data, whereas the similarity of circadian cortisol acrophases examined by objective cosinor methods throughout the year in women of different ages, in different populations, and on different continents (14), rests on dense data obtained every 20 minutes for 24 hours, Figure 3.

Figure 3



Results of fit of 24-h cosine curve to 24-h plasma cortisol profiles at 100-minute intervals. With data at 20-minute intervals, over 99% of series allow rejection of the no-rhythm assumption at the 1% level and all do so at the 10% level.

Discussion

With notable exceptions (3, 8, 37) (Table 1), much earlier discussion in the literature on cortisol in AN vs. CH is based on samples that were even more limited than those in the present study. Sampling limited to a single time-unspecified determination described as « fasting », with added determinations apparently stemming from response tests, can be cited (17). There is also a case when dense sampling is used only for computing a reliable mean (37). Others sampled twice a day, Table 1. It is on that basis that some authors discuss a deviant circadian variation, whereas still others feel that the variation is within the expectation for clinically healthy subjects. Hurd et al. (18) report that in 42% of their patients, serum corticosteroid concentration was elevated and circadian variation lost or reversed in 53%, and that weight loss was correlated with the urinary excretion of ketogenic steroids: the greater the weight loss, the higher the systemic steroid excretion. These authors refer to a discrepancy in AN between the serum concentration and the urinary excretion of corticosteroids, on the basis of data limited to

serum samples from 2 timepoints, in keeping with the practice of others (Table 1).

It is noteworthy that the most extensive sampling--every-20 or every 30-minute sampling (3, 8)--yielded the overall (macroscopic) impression of no deviant result. It is possible that the unaided eye, seeking to interpret small differences in pattern of variation (macroscopically) in a time plot, may often be in the same position as the eye unaided by histologic microscopy, trying to find a cell (macroscopically) in a tissue. Rhythmometric (microscopic) procedures such as the cosinors used herein provide objective point and (confidence) interval estimates of rhythms when the data are sufficiently dense. When the data are relatively sparse (for a rigorous individualized assessment), an imputed characteristic in AN, such as the amplitude-acrophase pair, may be interpreted by reference to a prediction region established on the basis of institutional sampling, in Figures 2a and 2b, or on the basis of international reference standards (Figure 2c).

It is also possible that other factors account for the difference in outcome. Further analyses are needed before one can conclude that the sparser sampling discriminates better and, if so, to determine to what extent alterations of waveform may be involved.

Whereas the finding of a statistically significantly different behavior as a function of the extent of weight loss experienced by the AN patients (those with the deviant acrophases showing, on the average, a greater deficit) is apparently original, it complements earlier focus by Gerner and Gwirtsman (12) upon the extent of weight loss in the light of dexamethasone suppression tests. The cortisol concentration after dexamethasone suppression was correlated with the percentage of ideal weight ($r = .35$); there was a trend for the cortisol concentrations of the 11 women who were less than 65% of their ideal weight to be higher than the concentrations of the 5 women who were 74% — 80% of their ideal weight (17.3 ± 2.2 versus 9.5 ± 2.1 μg/dl, $t = 2.12$, $p \sim 0.05$).

Differences in the circadian and circannual timing of the periodic production of aldosterone, cortisol, dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S) are noteworthy; they suggest the periodic operation of a 4-way switch in the pathway of adrenocortical steroid biosynthesis preferred at a given stage. Thus, in human beings, along the 24-hour scale, high values of aldosterone precede those of cortisol, with the latter preceding high DHEA, with high DHEA-S concentrations lagging behind all of these. Although the concentrations of these hormones

in blood are vastly different, their time relations, notably of DHEA-S, along the scale of a year as well as of a day may be important markers of enzymatic adrenocortical activities. The pineal may contribute to this timing, since it is already known that it rhythmically modulates (attenuates, leaves unaffected or amplifies) a pituitary tropic hormone (ACTH) effect upon adrenal corticosterone production (26-28). This modulation may be impaired if not absent in some patients with AN.

Deviant acrophases, such as those here noted for AN, can also be seen in our analyses of data obtained with a sampling similar to that here practiced on 3 out of 4 children with AN in Munich, Federal Republic of Germany (13), as well as in very young CH children (less than 1 year of age) in Miki, Japan (23). In CH children two years of age or older, the amplitude-acrophase pair is within the international reference standard for cortisol, computed with the amplitude as a percentage of the MESOR. It seems pertinent that a statistically significantly higher cortisol concentration in anorexia nervosa found earlier (3, 6, 8, 10, 11, 19, 36, 37) was shown by Zumoff et al. (37) to be associated with a lowering of adrenal androgen ($p < 0.05$). The DHEA-to-cortisol ratio representing the relative activities of pathways from 17-hyroxypregnenolone to DHEA vs. cortisol averaged less than half of the reference value in AN, while in relapse, whereas in partial remission, there was an increase in DHEA-to-cortisol ratio. The failure of DHEA concentrations to rise in response to ACTH in AN patients resembles the picture seen in pre-adrenarcheal children and is probably due to low activity of 17, 20 - lyase. What is physiological in pre-adrenarche may be pathological in AN. The lowered DHEA-to-cortisol ratio then, constitutes a hormonal parameter of ontogenic regression (34). Even earlier evidence that AN may represent a regression to pre-puberty was found by Boyar et al. (4), who report that the 24-h plasma luteinizing hormone secretory pattern was similar to that seen in pre-pubertal children.

Along the same line of thought, our analyses of systematic data from Japan by Onishi et al. (23) show that in the first year of life as compared to later in life, the timing of the circadian variation in cortisol is drastically different. One could postulate that some of the patients with AN might be regressed into the circadian pattern of very early childhood.

More than serum and even urinary cortisol will have to be determined for those who wish to look at the mechanisms of AN. In view of the failure of the mean to discriminate (in this study and in those of others) between patients with large and small deficits in body weight,

any endocrine focus upon AN will have to include an objective measure of timing, since an alteration of timing, established with the cosinor method herein, is also implied macroscopically by others, referring, e.g., to a reversed pattern, Table 1.

The data herein, limited to four samples a day, have served primarily for the comparison of patients with AN and subjects in CH, but they also achieve subgroupings as a first step by weight deficit. The question whether a change in cortisol acrophase, the discriminant found, and perhaps some change in a rhythm characteristic of another hormone (yet to be demonstrated) is an unspecific concomitant of AN, or rather an important co-determinant of the condition, is a topic for further research, to be planned in the light of this work. Our findings are in keeping with an alteration of cephalo-adrenal interaction (16). A direct pineal effect upon pituitary-adrenal interactions, rather than necessarily a hypothalamically-mediated pineal effect, has recently been demonstrated as a so-called feed-sideward (16, 26-28), apart from any feedbacks from the adrenal to the pituitary or hypothalamus. The pineal, in a rhythmic sequence, attenuates, leaves unaffected or amplifies the pituitary (or the ACTH) effect upon the adrenal in vitro (and thus in the absence of the hypothalamus) (16, 26-28). What is particularly pertinent to the findings of this study, under certain conditions, the pineal shifts a rhythm that, for one reason or another, is out of phase (26). Could this aspect of pineal modulation be deficient in AN? This possibility is a matter for further consideration to be explored with frequent sampling on the several variables that reflect not only the cortisol pathway but also competing pathways coordinating electrolyte and sex hormone metabolism in the adrenal itself and also in its superimposed and juxtaposed coordinators, the pituitary, hypothalamus and pineal, now known to interact in a time-dependent fashion and with important consequences in terms of body defense and behavior (16, 26-28).

Since the hormones involved in CH as a reference standard for AN exhibit a set of rhythmic changes with several frequencies, the work herein may be used to suggest that rather than being a vexing source of variability, the study of circadian, circannual and other rhythms can yield novel and useful parameters that thus far have discriminated between AN patients with large or small body weight deficit, pointing to mechanisms of cephaloendocrine interactions and suggesting the need to test in particular a possible alteration of a pineal feed-sideward upon pituitary-adrenal interaction. Answers to whether cephaloadrenal interactions constitute a determinant of AN will have to be explored with

strategically-placed cost-effective sampling (14) on the several variables implicated by the new finding of a quantitative change in timing of the cortisol rhythm, here interpreted in the light of the voluminous literature on adrenocortical function and steroids more broadly in AN, with only a citation (1, 2, 5, 20, 24, 31, 32, 34, 35, 38) to some other pertinent endocrine and metabolic studies.

Conclusion

Reliance in this paper, of necessity rather than choice, upon a less specific fluorometric method, as compared to a radioimmunoassay, forestalls definitive inferences. A set of radioimmunoassays on much denser data but on a different population (37) warrants the inference that serum cortisol in patients with anorexia nervosa and a certain extent of body weight loss, and in healthy subjects, is similarly timed. Whether or not this inference is to be extended to data from Warsaw, the present study, by being fluorometric and thereby picking up additional fluorogens, should prompt focus upon rhythms in substances other than cortisol that may also exhibit deviant characteristics in anorexia nervosa.

[1] *Institutional* in relation to *reference limits*, in the context of this paper, specifies 1) a population of women studied in Warsaw, 2) the fluorometric method used for cortisol determination and 3) a certain approach in determining body weight status for the diagnosis of anorexia nervosa. The Varsovian institutional reference standard differs from that in New York, also discussed in this paper, by all three criteria, i.e., geography and methodologies for cortisol determination and for assessing body weight loss. Whether any one or several of the three differences in possibly contributing to the results here found remains to be elucidated.

Riassunto

Variazioni del ritmo circadiano del cortisolo plasmatico possono indicare alterazioni della regolazione cefalo-ipotalamo-ipofisi-surrenalica in corso di anoressia nervosa (A.N.). Il cortisolo plasmatico è stato dosato con metodo fluorimetrico in 22 donne con A.N. e 18 donne clinicamente sane ai tempi 06, 12, 16 e 22. Sulla base dei gruppi i livelli medi di cortisolo erano significativamente più alti nel gruppo di A.N. in raffronto al gruppo di controllo, ma il MESOR non differisce nei pazienti con A.N. con il MESOR dei controlli sani. Un ritmo circadiano significativo è stato riscontrato in entrambi i gruppi con il metodo del « single cosinor ». Sono stati valutati i limiti di riferimento istituzionale per le donne sane come regioni di predizione al 90% per le misure di ampiezza/acrofase. Sei delle pazienti con A.N. hanno mostrato caratteristiche circadiane del cortisolo fuori dei limiti istituzionali di riferimento per il paio ampiezza/acrofase dei normali. Le pazienti con A.N. le cui acrofasi di cortisolo erano fuori dei limiti di riferimento istituzionale avevano i più gravi deficit ponderali. Il tempo di deviazione, misurato con l'ampiezza/acrofase pesato, è in accordo con altri comportamenti regressivi riscontrati nelle pazienti con A.N.

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