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Differences between Young and Elderly Subjects in Seasonal and Circadian Variations of Total Plasma Proteins and Blood Volume as Reflected by Hemoglobin, Hematocrit, and Erythrocyte Counts

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Circadian and seasonal rhythms in total plasma proteins were documented in healthy young men (around 24 years old), and in elderly subjects (both sexes), including senile-dementia patients in their eighties. The concentration of plasma proteins within a given group changed predictably (7–13%), depending on the hour of sampling and the season. Concentrations decreased noticeably around 04:00 h, then peaked around 08:00 h (shortly after waking). The 24-h mean concentrations of total plasma proteins were lower in the elderly groups than in the young men. But the seasonal variations of the 24-h mean values were strikingly larger in the elderly groups (7–8 g/L) than in the young men (2–5 g/L). Moreover, the circadian profiles of plasma proteins differed from the profiles of hematocrit, hemoglobin, and erythrocyte counts. Evidently, circadian variations of blood volume may not be the only element accounting for the variations of plasma protein concentrations. We suggest that the rhythms in plasma protein concentrations be taken into account when reference values are set. Circadian and seasonal variations in plasma proteins may also significantly affect the transport and binding of drugs, especially in the aged.

Additional Keyphrases: *variation, source of · age-related effects · pharmacokinetics · senile dementia · reference values · biological rhythms*

Plasma proteins play an important role in physiology and pharmacology, especially in binding various molecules, including a large variety of hormones and drugs. Knowledge of the circadian and seasonal variations of plasma proteins is doubly useful: first, because they can affect the interpretation of the concentrations of the bound and unbound fractions of a physiological or pharmacological agent; second, because they should be taken into account in designing a therapeutic protocol that optimizes the tolerance and expected effects of drugs and diminishes their side effects. Age is also an important factor to consider, recent studies having established that aging often modifies the expression of biological rhythms (*1–4*).

Our aim in this present study was to document the seasonal variations in circadian changes in total plasma proteins concentration in young and elderly subjects. The rhythmic variations of hematocrit, erythrocyte count (RBCC), and hemoglobin concentration were also documented as indexes of blood volume.

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Subjects, Materials, and Methods

Subjects. Seasonal changes in circadian rhythms were documented during the same 24-h intervals in January, March, June, and October. We assembled the following four groups of subjects in Paris, France, for a transverse study: (*a*) seven apparently healthy young men (medical students), ages 19 to 31 years (mean ± SD, 24.0 ± 3.9 years at the beginning of the study); (*b*) six elderly men, mean age 75.3 (SD 6.6) years; (*c*) six elderly women, mean age 78.2 (SD 9.1) years; (*d*) six elderly patients with senile dementia, two men and four women, mean age 81.1 (SD 8.6) years.

The protocol of investigation, and details on the selection of the 18 elderly subjects (physical and psychiatric evaluation with reference to the well-known polypathology of the aged) have been published previously (*1*). Positive factors for the selection of subjects included regularity of professional (in young subjects) and social routines, eating habits, and sleep schedule. The subjects had a regular social routine, with lights turned on at 07:00 h (± 1 h) and off at 21:00 h (± 1 h) for the elderly and at 23:00 h (± 1 h) for the young. Meals, unrestricted in food and water intake, were taken at fixed times, i.e., by the aged at 08:15, 12:15, and 19:15 h (± 15 min), and by the young at 08:00, 12:30, and 20:00 h (± 1 h).

None of the young subjects had taken any medication for at least three months before the study. Those of the elderly who were taking medication, mostly small doses of tranquilizers or hypnotics before sleep, were not given their medications for at least five days before each of the tests.

Design of the investigation. On test days venous blood samples were drawn without inducing stasis (*5*) at fixed 4-h intervals during a 24-h period beginning at 07:45 h. To standardize the experimental conditions, i.e., the relationship between posture and physical activity and blood volume (*6, 7*), we had the subjects rest recumbent for 15 to 30 min before each blood sample was taken, which is considered long enough for the shift in fluids to be fairly complete (*6–8*). Vacutainer Tubes containing 0.1 mL of 0.37 mol/L EDTA K_3 (Becton Dickinson-SA, Grenoble, France) were used in collecting the 10-mL blood specimens.

RBCC, hemoglobin, and hematocrit were determined without delay with an automated blood cell analyzer (Model S; Coultronics-France SA, Margency, France). Respective CVs were 1.5, 1.0, and 1.5% for RBCC of $5 \cdot 10^{12}$ cells/L, hemoglobin concentration of 0.744 mmol/L, and hematocrit of 0.4.

The blood specimens to be used for determination of total proteins were centrifuged and the plasma was separated from the cells without delay and immediately processed. Total plasma proteins were determined manually by the biuret colorimetric method with potassium iodide (Ames, Paris, France) and by measuring the absorbance at 539 nm

(we used an LKB 2074 Clinicon spectrophotometer, LKB Instruments-SA, Orsay, France). Normal and pathological commercial control sera were included in each series of analyses. CVs were 1.3 and 1.8% for protein concentrations of 67.9 and 57.6 g/L, respectively.

Statistical analysis. First we plotted the data as a function of time (mean ± SEM), to show daily and seasonal changes. We tested the significance of peak–trough differences by Student's *t*-test. Circadian and seasonal variations were submitted to analysis of variance (three-way ANOVA).

Results

Circadian Rhythms

Hematocrit, erythrocyte count, hemoglobin. The circadian patterns of these three variables were the same in each group of subjects during each season (Figure 1). The analysis of variance validated the lack of time/season interaction (Table 1). The lowest values were found at either 23:45 h or 03:45 h, the highest at 07:45 h. As a rule, the percent drop (circadian peak value minus circadian trough value × 100 divided by circadian peak value) was lower in young men (Table 2), i.e., around 5%, than in the elderly groups (7–13%), thus largely beyond the range of analytical variations. For each variable and during each season the circadian mean values were markedly lower in elderly groups (Table 2). The analysis of variance validated a statistically significant group-effect and a significant circadian rhythm (time-effect) for each variable, but no group/time interaction was observed (Table 1).

Plasma total proteins. Differences between the profiles of plasma proteins (Figure 2) and the profiles of hematocrit, RBCC, and hemoglobin (Figure 1) were more marked than were the differences between the profiles of hematocrit, RBCC, and hemoglobin. The lowest protein concentrations for each group of subjects were found around 03:45 h and the highest, as a rule, between 07:45 h and 11:45 h. The percent drop (Table 2) differed between age groups (8–11%) and, within a given group, according to the season. These circadian decreases also exceeded the analytical variations. Circadian means were about 7 g/L lower in elderly subjects than in young men, except in October (2 g/L). The analysis of variance validated a statistically significant group-effect and a significant circadian rhythm (time-effect) for plasma total proteins but no time/season interaction was observed (Table 1).

Seasonal Rhythms

The seasonal patterns of plasma proteins, RBCC, hematocrit, and hemoglobin are shown in Figure 3. Large differ-

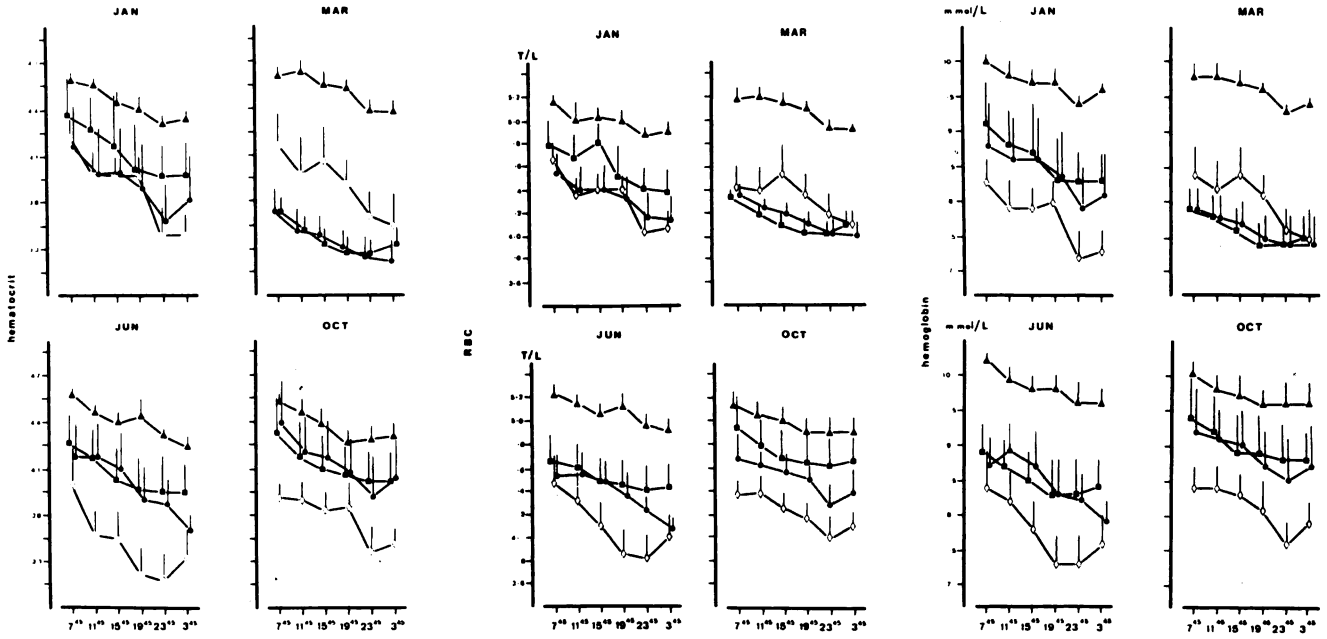

Fig. 1. Circadian patterns of (*A*) hematocrit, (*B*) erythrocyte (RBC) count, and (*C*) hemoglobin concentration in seven young men (▲), six elderly men (■), six elderly women (●), and six elderly demented patients, two men and four women (◇)

Bars indicate 1 SEM. Clock time is shown on the *x*-axis

Table 1. Analysis of Variance for Circadian and Seasonal Rhythms in Plasma Total Proteins, Hemoglobin, Hematocrit, and Erythrocyte Count (RBCC)

Variable (and degrees of freedom)	Proteins		Hemoglobin		Hematocrit		RBCC	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Group (3)	72.59	<0.001	122.6	<0.001	83.25	<0.001	136.8	<0.001
Time (5)	43.58	<0.001	9.17	<0.001	13.76	<0.001	13.72	<0.001
Season (3)	19.87	<0.001	16.35	<0.001	8.96	<0.001	5.32	<0.002
Season/group (9)	12.47	<0.001	5.14	<0.001	9.50	<0.001	6.68	<0.001
Time/group (15)	1.40	>0.14	0.23	>0.99	0.40	>0.98	0.40	>0.98
Season/time (15)	0.30	>0.99	0.15	>0.99	0.16	>0.99	0.14	>0.99

Table 2. Twenty-Four-Hour Means (and SEM) of Plasma Total Proteins, Hemoglobin, Hematocrit, and RBCC in Various Groups of Subjects

Groups and time of sampling	Proteins, g/L		Hemoglobin, mmol/L		Hematocrit		RBCC, 10^{12} cells/L	
	Mean	% drop[a]	Mean	% drop	Mean	% drop	Mean	% drop
Young men								
January	71.6 (0.7)	10.6***	9.6 (0.1)	5.9**	0.444 (0.003)	6.1**	5.01 (0.04)	5.0*
March	72.9 (0.6)	8.7**	9.6 (0.1)	5.1*	0.452 (0.003)	5.4*	5.08 (0.04)	5.4**
June	71.0 (0.5)	8.1***	9.8 (0.1)	6.7*	0.440 (0.003)	7.2**	5.07 (0.04)	5.8*
October	73.5 (0.5)	7.6***	9.7 (0.1)	3.8	0.437 (0.004)	5.5*	4.92 (0.05)	4.5
Elderly men								
January	68.2 (0.7)	8.9**	8.6 (0.2)	9.1	0.412 (0.009)	9.2	4.58 (0.08)	8.7
March	66.1 (0.9)	7.0*	7.6 (0.1)	7.0	0.357 (0.004)	7.5*	4.13 (0.04)	6.9*
June	65.5 (0.7)	7.8*	8.5 (0.2)	5.6	0.405 (0.008)	7.5	4.49 (0.09)	5.8
October	72.2 (0.7)	7.3*	9.0 (0.2)	6.5	0.411 (0.008)	6.9	4.72 (0.09)	6.5
Elderly women								
January	66.3 (0.9)	12.5**	8.4 (0.2)	10.4	0.390 (0.010)	11.8	4.33 (0.08)	8.8
March	68.0 (0.7)	5.3	7.6 (0.1)	7.0	0.356 (0.005)	8.8	4.15 (0.04)	7.8*
June	63.7 (0.9)	11.8**	8.4 (0.2)	11.2	0.399 (0.008)	11.5	4.37 (0.07)	10.1
October	72.0 (0.9)	10.4**	8.9 (0.2)	7.9	0.414 (0.009)	10.7	4.51 (0.07)	8.3
Elderly demented								
January	65.8 (0.8)	8.1	7.8 (0.1)	13.2*	0.389 (0.006)	14.7**	4.33 (0.06)	5.6*
March	66.6 (1.1)	17.7***	8.1 (0.2)	11.0	0.392 (0.008)	12.7	4.40 (0.08)	9.7
June	64.1 (0.7)	11.9**	7.8 (0.2)	12.3	0.364 (0.007)	15.9	4.00 (0.09)	14.3
October	71.2 (0.7)	9.0**	8.1 (0.1)	14.7	0.377 (0.005)	8.7	4.21 (0.06)	8.9

Significance of decrease (Student's *t*-test): $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

[a] Percent drop = [(higher circadian concentration − lower circadian concentration)/higher circadian concentration] × 100.

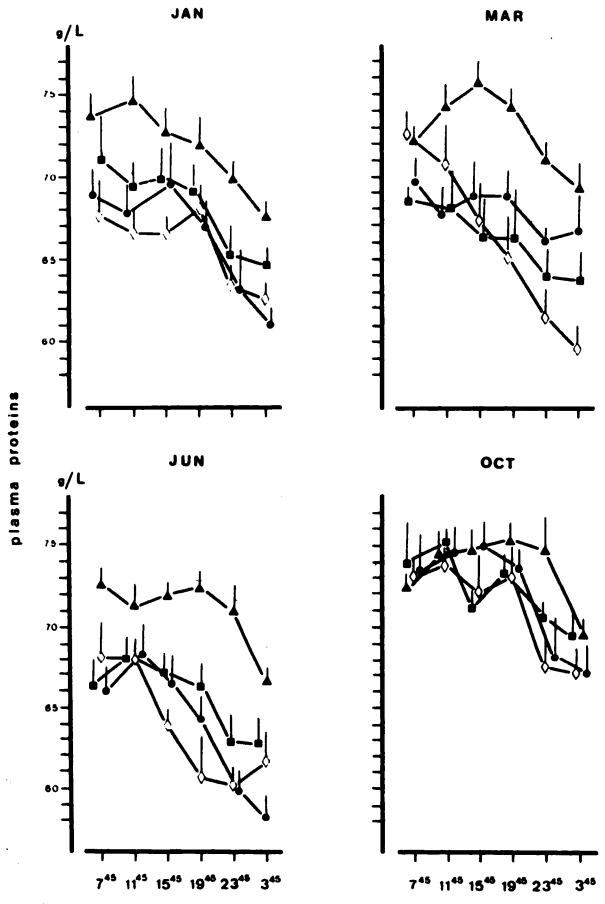

Fig 2. Circadian patterns of plasma protein concentrations

Subjects and symbols as in Figure 1

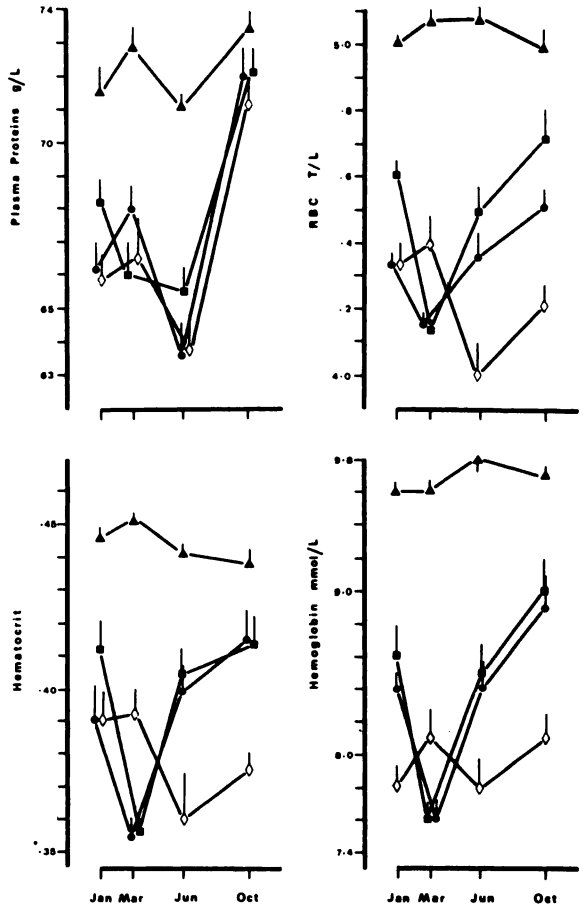

Fig 3. Seasonal patterns of plasma proteins, erythrocyte (RBC) count, hematocrit, and hemoglobin

Subjects and symbols as in Figure 1

ences appeared to be related to the subject's age and (or) mental health. For any variable, seasonal changes were more marked in the elderly groups than in the young men. In most cases, there were seasonal peaks in October and seasonal troughs in March–June in elderly groups. The seasonal percent drops were much smaller in the young men (2.0–3.4%, depending on the variable) than in the elderly groups (3.7–15.5%).

The yearly mean (± SEM) of plasma proteins was 67.4 ± 1.3 g/L in the elderly men, approximately 4 g/L less than in the young men (71.7 ± 0.5 g/L). Yearly means of hematocrit, hemoglobin, and RBCC were lower in the elderly subjects than in the young men: 9–16, 12–19, and 10–16% lower, respectively, depending on the group of elderly.

Discussion

We found differences in the circadian rhythmicity of plasma proteins on the one hand, and hematocrit, hemoglobin, and RBCC on the other hand. Whereas the profiles of these last three variables were identical whatever the group of subjects or season, the circadian pattern of plasma proteins varied over the year and according to the group of subjects studied. This indicates that the circadian rhythms of blood volume may not be the only component accounting for the rhythms in plasma protein concentrations. Circadian rhythms in protein synthesis have been documented in a set of species of monocellular eukaryotes (*9*), and the circadian rhythm of the synthesis of protein by the rat liver has been described (*10*). The positive or negative balance between synthesis and degradation of plasma proteins, in addition to the variations resulting from the rhythm of volemia, might thus explain the observed discrepancy between the circadian patterns of plasma proteins and those of hematocrit, hemoglobin, and RBCC in young and elderly human subjects.

This study clearly showed that (*a*) the concentrations of plasma proteins underwent predictable changes (7–13%) depending on the hour of sampling; (*b*) these changes, which were much larger than the analytical variability, were found in all groups of subjects and in any season; (*c*) the locations of the seasonal peak values in young men agreed with those reported by Statland et al. (*11*) and Reinberg et al. (*12*); and (*d*) the 24-h mean concentrations of plasma proteins showed larger seasonal variation in elderly groups (7–8 g/L) than in young men (2.5 g/L). The last finding may be attributable to age-induced differences in the seasonal regulation of blood volume and (or) to age-induced differences in the synthesis–catabolism balance of plasma proteins. The possibility of larger seasonal variations in physical activity on the part of elderly subjects than for young men might also account for this phenomenon.

These data on the circadian and seasonal rhythms agree well with those we previously obtained with the same groups of subjects for concentrations of free cortisol in plasma (*2*). The higher concentrations of free cortisol in elderly subjects than in young men can be explained by the lower concentration of plasma proteins in the elderly. Moreover, the 24-h mean value for plasma free cortisol was higher in June than in other seasons, which corresponds to the seasonal trough for plasma proteins.

We conclude that the seasonal rhythm in the concentration of plasma proteins should be taken into account when one is determining the so-called reference values for plasma proteins, especially in the aged, given the large variations noted for the elderly groups in this study. These variations should also be considered when designs of therapeutic protocols are being evaluated, to optimize the tolerance and expected effects of drugs and minimize their side effects, because the circadian and seasonal fluctuations of plasma proteins might produce significant variations in the transport and binding of drugs, especially in the aged. We also emphasize that the subjects exhibited a rather large nocturnal decrease in plasma proteins (to around 04:00 h) and a peak concentration around waking (08:00 h).

To summarize, we found the following maximum circadian and seasonal variations: the lowest vs highest hourly concentration of plasma proteins in the year differed by as much as 9 g/L in the young men, 12 g/L in the elderly men, 14 g/L in the elderly demented subjects, and 17 g/L in the elderly women of our study. Such variations are of interest both for the concept of reference values (according to the time of sampling, the age of the subject, and the season of the year) and for bioclinical interpretation.

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