Twenty-Four–Hour Variation in Serum Leptin in the Elderly

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To investigate the possibility that the aging process may affect the diurnal variation in serum leptin in humans, serum leptin levels were measured by a sensitive radioimmunoassay method in 12 elderly (aged 72 to 87 years) and 10 middle-aged (35 to 50 years) lean male subjects. Fasting blood samples (4 mL) were drawn at 8:00 AM, and then every 4 hours until 10:00 PM and every 2 hours from 12:00 midnight to 8:00 AM of the next morning. Circadian rhythmicity analysis was performed using the cosinor method. In elderly subjects, serum leptin levels showed a significant diurnal rhythm, which was similar to that observed in controls. Single cosinor analysis showed a significant rhythm in eight of 12 elderly subjects and in all middle-aged subjects but one. Compared with middle-aged subjects, similar mesor mean values (7.8 ± 1.0 *v* 8.1 ± 0.8 ng/mL) but a decreased amplitude (1.4 ± 0.3 *v* 2.3 ± 0.2 ng/mL) and an earlier acrophase (11:56 PM *v* 2:04 AM) were observed in the elderly. The data demonstrate that the diurnal variation in serum leptin is generally preserved in the elderly. However, the amplitude of leptin diurnal excursion undergoes a reduction with advancing age. It can be speculated that the blunted diurnal variation in serum leptin observed in the elderly may result in an alteration of the afferent signal in the adipose tissue–central nervous system homeostatic loop.

PREVIOUS INVESTIGATIONS have demonstrated that leptin, the protein secreted from adipose tissue[1] that regulates food intake[2] and energy expenditure,[3,4] undergoes diurnal variation in animals[5] and humans.[6] In normal subjects, serum leptin levels are maximal between midnight and the early morning hours and minimal around noon to midafternoon.[6,7] A similar 24-hour leptin secretory pattern has been shown in obese patients with and without non–insulin-dependent diabetes mellitus[6] and in female athletes with a normal menstrual cycle,[8] but not in young females during weight gain,[9] amenorrheic athletes,[8] and patients with anorexia nervosa.[10,11]

At present, no data are available regarding the diurnal variation in the serum leptin level in elderly subjects. However, it is well known that the aging process induces variations in the body composition[12] and the hormonal milieu,[13] which have been shown to be important contributors to the regulation of adipocyte *ob* gene expression[14] and, consequently, the morning leptin serum level. To ascertain whether an age-related change of the diurnal variation in serum leptin can occur in man, the 24-hour variation in the serum leptin level was determined in a group of elderly subjects compared with a group of normal middle-aged subjects.

SUBJECTS AND METHODS

Subjects

The study was performed in 12 elderly men (aged 72 to 87 years) and 10 middle-aged men (35 to 50 years) matched for body mass index (BMI) and hospitalized for the purpose of the study. All subjects were in good condition, and a physical examination and laboratory tests did not reveal hepatic, renal, or endocrine-metabolic diseases. None of the subjects were obese (BMI > 27.3 kg/m^2 according to the National Institutes of Health Consensus Development Panel,[15] none used medications known to alter carbohydrate metabolism, particularly thiazides, β-blockers, or psychotropic medications interfering with hypothalamic-pituitary function, and none smoked tobacco or abused alcohol. Informed consent was obtained from each subject.

Experimental Procedure

Weight and height were measured using a standard beam-balance. The BMI was determined as the weight in kilograms divided by the height in meters squared. The skinfold thickness of the chest, abdomen, and thigh was measured using a Lange caliper, and percent body fat (%BF) was calculated from these values and the subject's age using a gender-specific regression equation validated by comparison to underwater weighing.[16]

Evaluation of the 24-hour variation in serum leptin was performed 1 to 2 days after hospital admission in both the elderly and middle-aged subjects.

During the study period, each subject stayed in a single room and received regular hospital meals: breakfast at 8:00 AM (11 g protein, 47 g carbohydrate, 8 g lipid, 300 cal), lunch at 12:00 noon (45 g protein, 163 g carbohydrate, 26 g lipid, 1,140 cal), and dinner at 7:00 PM (40 g protein, 131 g carbohydrate, 34 g lipid, 1,047 cal). No snacks were provided between meals. The lights were turned off at 10:00 PM and the patients were allowed to sleep. At 7:00 AM on the study day, an intravenous catheter was inserted into an antecubital vein. It was not necessary to prevent occlusion of the catheter by either saline or heparin infusion, and patients were free to perform their daily routine. Fasting blood samples (4 mL) were drawn at 8:00 AM, and then every 4 hours until 10:00 PM and every 2 hours from 12:00 midnight to 8:00 AM the next morning.

Hormone Assay

Leptin serum concentrations were determined by a radioimmunoassay method using reagents supplied as a kit by DRG Instruments (Marburg, Germany). The lowest amount of leptin detectable in serum was 0.5 ng/mL. The within- and between-assay mean coefficients of variation were 3.9% and 4.3%, respectively.

Statistical Analyses

Leptin values for each sample were expressed as a percent of the 24-hour individual mean. The single and mean cosinor methods were used to detect and quantify the 24-hour secretory rhythm by estimation of the following parameters: mesor (the rhythm-adjusted mean), amplitude (half of the total variability of the considered period), and acrophase (peak time of the best-fitting cosine function used to approximate all data). Differences between group means were calculated by two-tailed Student's *t* test for unpaired observations. Correlations between the variables were assessed by simple and multiple linear regression analysis Aging was coded as 1 or 2, with the lower value

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indicating the elderly. Acrophases recorded in the two groups were statistically compared after transforming the hours in angles. Data are expressed as the mean ± SEM.

RESULTS

The two groups were well matched for BMI. The %BF in the elderly tended to be higher but was not statistically different compared with the %BF in middle-aged subjects (Table 1).

The profiles of diurnal variation in serum leptin recorded in the elderly and middle-aged subjects are listed in Table 1 and illustrated in Fig 1.

Leptin fasting serum levels were comparable in the elderly (6.9 ± 0.9 ng/mL) versus middle-aged subjects (7.5 ± 0.7 ng/mL), and for both groups, the levels significantly ($P < .01$ to .05) correlated with the BMI ($r = .874$ and .854 in elderly and middle-aged subjects, respectively) and the %BF ($r = .603$ and .748 in elderly and middle-aged subjects, respectively).

The mean cosinor analysis of the 24-hour leptin variation showed a significant ($F = 7.362$; $P < .01$) rhythm in the elderly, similar to that observed in middle-aged subjects ($F = 17.701$, $P < .01$). Single cosinor analysis showed a significant rhythm in eight of 12 elderly subjects and in all but one middle-aged subject. The mesor recorded in the elderly (7.8 ± 1.0 ng/mL) was similar to that recorded in middle-aged subjects (8.2 ± 0.8), and in both groups, it significantly ($P < .01$ to .05) correlated with the fasting leptin level ($r = .972$ and .883 in elderly and middle-aged subjects, respectively), %BF ($r = .625$ and .834 in elderly and middle-aged subjects, respectively), and BMI ($r = .890$ and .946 in elderly and middle-aged subjects, respectively). The amplitude was significantly lower ($P < .025$) in the elderly (1.4 ± 0.3 ng/mL) compared with middle-aged subjects (2.3 ± 0.2 ng/mL). In contrast to middle-aged subjects, in whom there was a positive correlation between the amplitude and fasting leptin ($r = .659$. $P < .05$), BMI ($r = .903$, $P < .01$), and %BF ($r = .757$, $P < .01$), in the elderly, the amplitude did not correlate with the fasting leptin level ($r = .486$, $P > .05$), BMI ($r = .555$, $P > .05$), and %BF ($r = .548$, $P > .05$). Elderly subjects showed an acrophase at 11:56 PM, which was significantly ($P < .05$) earlier in comparison to that recorded in middle-aged subjects (2:04 AM).

By multiple linear regression analysis, a significant contribution of aging in determining the amplitude and acrophase but not the mesor of leptin's diurnal rhythm was also observed (Table 2).

DISCUSSION

Our data demonstrate that a significant diurnal variation in the serum leptin level was generally preserved in the elderly, being detectable in the majority (66%) of the subjects studied. However, compared with that recorded in the middle-aged subjects, the diurnal variation of serum leptin observed in the

Table 1. Clinical Characteristics and Single and Mean Cosinor Analyses of Diurnal Variation in Serum Leptin in Elderly and Middle-Aged Subjects

Subject No.	Age (yr)	BMI (kg/m²)	%BF	Fasting Leptin (ng/mL)	Mesor (ng/mL)	Amplitude (ng/mL)	Acrophase (h)	Cosinor P
Elderly								
1	75	24.4	24.5	6.5	6.7	0.8	3.29	<.05
2	83	22.2	21.8	5.2	4.4	0.5	1.49	>.05
3	77	21.7	25.7	4.0	4.7	1.2	0.25	<.01
4	78	23.8	26.3	7.0	7.1	1.4	2.13	<.01
5	72	26.2	31.9	14.0	15.6	1.9	0.52	<.05
6	80	25.2	27.4	10.8	12.3	3.0	22.14	<.05
7	87	24.3	23.9	8.8	8.9	0.6	18.49	>.05
8	78	19.8	26.8	2.2	2.9	0.9	20.22	<.01
9	76	23.8	22.9	5.8	7.0	1.0	0.22	>.05
10	72	24.9	29.3	8.0	9.9	3 0	1.44	<.01
11	80	24.5	22.0	5.8	7.9	2.3	23.21	<.05
12	80	22.9	21.3	5.3	6.4	0.8	17.31	>.05
Mean	78.17	23.64	25.34	6.95	7.82	1.45*	23.56*	<.01
SEM	1.24	0.50	0.93	0.91	1 01	0.26		
Middle-aged								
1	48	24.8	24.3	9.3	9.4	2.1	2.10	>.05
2	46	26.3	25.4	9.0	10.9	3.4	2.14	<.05
3	36	23.8	22.9	9.0	7.4	2.2	3.31	<.05
4	50	26.2	22.3	9.5	11.1	2.9	1.15	<.01
5	35	21.7	20.2	5.5	5.4	1.7	0.44	<.05
6	44	20.1	19.4	2.8	3.7	1.5	2.30	<.05
7	45	23.2	22.8	7.9	7.5	2.1	1.47	<.01
8	46	21.8	22.0	7.4	7.2	1.4	3.58	<.01
9	48	25.6	30.0	9.6	12.1	3.4	1.21	<.05
10	44	23.5	20.0	5.4	6.8	2.3	2.12	<.01
Mean	44.20	23.70	22.93	7.54	8.15	2.31	02.04	<.01
SEM	1.57	0.66	0.90	0.72	0.85	0.22		

*$P < .05$ *v* middle-aged.

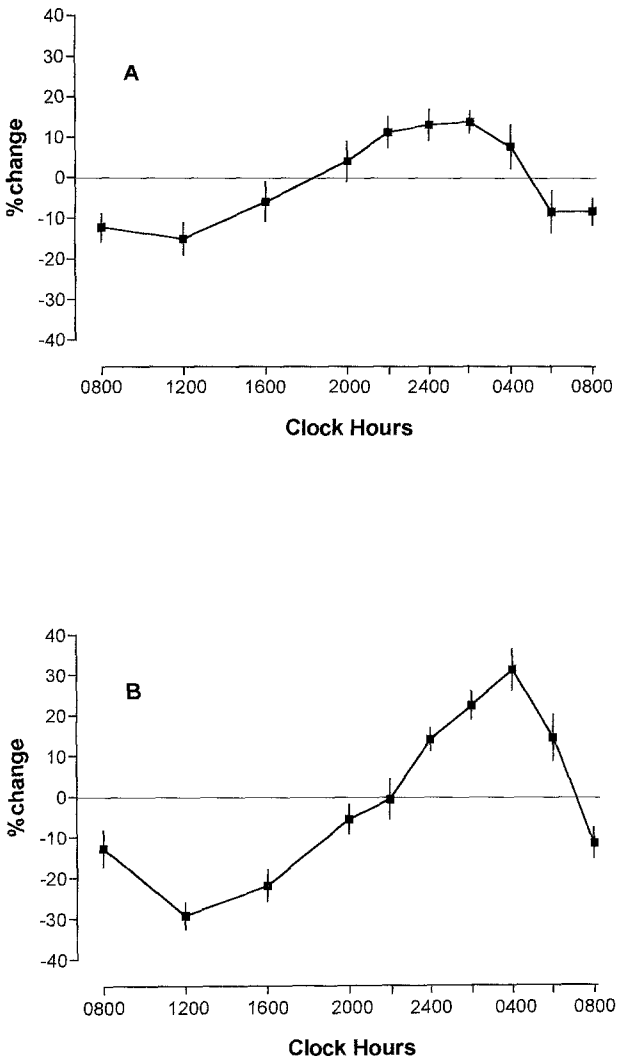

Fig 1. Percent change (mean ± SEM) from the 24-hour mean for serum leptin values recorded in 12 elderly (A) and 10 middle-aged (B) subjects. Hours are 8:00 AM, 12:00 noon, 4:00 PM, 8:00 PM, 12:00 midnight, 4:00 AM, and 8:00 AM, respectively.

elderly shows some differences consisting of an earlier acrophase and a decreased amplitude. Furthermore, in contrast to the middle-aged subjects, in whom a positive correlation among the diurnal amplitude and BMI, %BF, and fasting leptin level was recorded, in the elderly subjects, the amplitude of diurnal variation in serum leptin did not correlate with these parameters. However, when the whole population was statistically considered, a negative linear correlation between aging and the amplitude of diurnal variation in serum leptin, but not between aging and mesor or fasting leptin levels, was recorded.

It is known that a decrease in lean mass and an increase in fat mass occur in humans as a consequence of aging.[12] Furthermore, it has been demonstrated that the increased fat mass in humans is associated with a blunted diurnal excursion and dampened pulsatility of leptin secretion.[9,17] Thus, the possibility that an age-related increase in fatness may influence the diurnal variation in the serum leptin level in the elderly cannot be ruled out. However, in the elderly of our study, the amplitude of diurnal variation in serum leptin was lower at any %BF, and when all subjects were statistically considered, aging appears to be the major determinant of the amplitude of diurnal variation in serum leptin. This correlation does not appear to be due to the colinearity existing among fasting leptin, %BF, and BMI, since the values for these variables recorded in the elderly were similar to those recorded in middle-aged subjects. Indeed, in a recent study, a loss of the physiological correlation between leptin secretion and body fat content with advancing age in humans has been reported.[18] Furthermore, a relative increase in body fat cannot explain the earlier acrophase in the elderly, since a similar timing of the diurnal peak and nadir of the leptin serum level in lean and obese subjects has always been observed.[6,17]

The mechanisms through which age may affect the diurnal variation in serum leptin are presently unknown. A loss of circadian rhythmicity and a diminution of the amplitude of circadian rhythms are characteristic of aging.[19] This has been attributed to an age-related impairment of pacemaker output and/or a partial inability of effector systems to respond to a normal pacemaker input.[20] Thus, the possibility that the diurnal variation in serum leptin may also be influenced by the age-related decrease in pacemaker output and/or a reduced ability of adipocytes to respond to normal physiological stimuli may be suggested. It is known that in many elderly individuals, there is a decrease of several variables associated with leptin secretion,[21,22] such as the body temperature[23] and the cortisol and melatonin[24] circadian rhythms, and the nocturnal growth hormone secretory peak is always reduced or even absent.[25] Furthermore, it has been demonstrated that in the elderly, adipocytes are less sensitive to catecholamines[26] and insulin,[27] which seem to play an important role in modulating leptin secretion.[21,22]

The pathophysiological significance of the reduced diurnal variation in serum leptin levels remains to be established. However, on the basis of evidence suggesting that leptin is the messenger of metabolic signals from the adipose tissue to neural structures regulating food intake,[28] we may speculate that the blunted diurnal variation in serum leptin may result in an alteration of the afferent signal in the adipose tissue–central nervous system homeostatic loop in the elderly.

Table 2. Multiple Linear Regression Analysis of Variables Associated With Parameters of Diurnal Variation in Serum Leptin in 22 Subjects

Dependent Variable	Independent Variables	β Coefficient (mean ± SEM)	*P*
Mesor (ng/mL)	Fasting leptin (ng/mL)	0.614 ± 0 140	.0004
	%BF	0.164 ± 0.078	.0449
	BMI (kg/m²)	0.586 ± 0.182	.0051
	Aging (A = 1, B = 2)	−0.323 ± 0.428	.4609
Amplitude (ng/mL)	Fasting leptin (ng/mL)	−0.154 ± 0.089	.1008
	%BF	0.133 ± 0.049	.0147
	BMI (kg/m²)	0.372 ± 0.115	.0049
	Aging (A = 1, B = 2)	−1.240 ± 0.271	.0003
Acrophase (h)	Fasting leptin (ng/mL)	−8.270 ± 19.442	.6759
	%BF	9.763 ± 10.790	4607
	BMI (kg/m²)	7.021 ± 25.339	.7902
	Aging (A = 1, B = 2)	−136.908 ± 59 443	.0342

Abbreviations: A, 12 elderly; B, 10 middle-aged.

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