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Endogenous Melatonin Levels and the Fate of Exogenous Melatonin: Age Effects

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This study examines the range of serum melatonin concentrations that occur among young and older adults, and tests the effects of orally administered melatonin on the serum and saliva concentrations of the hormone. Healthy volunteers (20–36 per study), aged 20–73 years, were divided into two groups on the basis of age (29.2 ± 6.5 and 60 ± 8.8 years). For study 1: Serum melatonin levels were measured at 15 to 60 min intervals over a 25 h period using a radioimmunoassay. For study 2: serum and saliva melatonin levels were measured before and at intervals after the administration of a 0.3 mg dose of melatonin at 11.00 h. The younger subjects had significantly higher peak endogenous melatonin concentrations (± SD) and greater inter-individual variability (100.9 ± 48.6 pg/ml) than the older subjects (34.5 ± 15.4 pg/ml). Mean melatonin levels following treatment with the hormone tended to be higher and were significantly more variable among the group of older volunteers (254.5 ± 145.7) than among the younger group (170.2 ± 22.0 pg/ml). We conclude that although the peak endogenous serum melatonin levels are lower in elderly adults, the increment in serum melatonin levels induced by a low oral dose of the hormone is greater and more variable among people over 48 years old.

MELATONIN, a pineal hormone (1,2), promotes sleep onset (3,4) and affects the timing of various circadian and annual rhythms in humans and other animals (5). In humans, circulating melatonin concentrations are about 1–2 orders of magnitude higher at nighttime than during the day (6), peaking in the early morning (1–5 a.m.) and declining thereafter. Circulating melatonin is inactivated in the liver where it is converted to 6-hydroxymelatonin and then conjugated with sulfate or glucuronide and excreted in the urine. Circulating melatonin levels reportedly decrease with physiologic aging (7–10).

The initial studies on the consequences of melatonin administration to humans (11) revealed that pharmacologic doses of the hormone produce a soporific effect; this effect was repeatedly confirmed in subsequent studies (3,4,12,13). Recently, physiological oral doses of the hormone, which elevate serum melatonin concentrations only to levels normally occurring nocturnally, were also shown to promote sleep onset and sleep maintenance (14–16). These observations, and other studies showing that similar doses of the hormone can alter the phase of circadian rhythms (17), probably underlie the current widespread use of melatonin to promote sleep or to treat jet lag. Therefore, it is important to understand the significance of variations in blood melatonin levels and the changes in circulating levels that follow melatonin administration, particularly in populations that may have very low or very high endogenous levels.

Accordingly, we have examined basal serum melatonin levels and the effects on these levels of a low oral dose of melatonin (0.3 mg) in a group of healthy young adults and in a group of older adults. We also examined the possibility that salivary melatonin measurements might provide a reliable index of circulating melatonin concentrations in the two populations.

Methods

All of the volunteers participating in these studies underwent a physical examination at the Massachusetts Institute of Technology (MIT) Clinical Research Center (CRC). Their urine and blood analyses were within the normal range. The experimental protocol and the subject's consent form were approved by the MIT Committee on the Use of Humans as Experimental Subjects. The subjects gave informed consent and reported that they were drug-free for at least 2 weeks prior to entering the study. The volunteers were asked to refrain from alcohol or caffeine consumption for 24 h prior to each test session.

Study 1

Two groups of 18 subjects participated in this study. Group I included 10 males and 8 females, with an age range of 20–43 years, and a mean age (± *SD*) of 29.2 ± 6.5 years. Group II contained 9 males and 9 females with an age range of 49–73 years, and a mean age (± *SD*) of 60 ± 8.8 years. The subjects' habitual bedtimes were identified on the basis of their self-reports. Subjects were admitted to the CRC for a 25 h period of blood sampling which started at 09.00 h. A catheter with a saline lock was established in the subject's forearm vein for withdrawing blood samples. Blood samples (2 ml each) were collected at 15-min intervals from 09.00 h to 11.00 h, hourly from 11.00 h to 19:00 h, at 15 min intervals from 19:00 h to 23:00 h, at 30-min intervals from 23:00 h to 05:00 h, and at 15-min intervals from 05:00 h to 09:00 h. The frequency of blood sampling was determined by the anticipated rate of changes in circulating melatonin levels, which is greater at the onset and offset of nocturnal melatonin secretion. Environmental illumination levels were controlled. Between 19.00 h and 22.00 h light intensity was held at 50 lx, between 22.00 h and 07.00 h at less than 10 lx, and after 07.00 h at 50 lx.

Study 2

Twenty of the subjects who participated in study 1 also participated in study 2. Two groups, each containing 10 individuals, were studied: group I included 6 males and 4 females, 20–32 years old (mean ± *SD*, 25.9 ± 4.7 years); group II included 4 males and 6 females, 49–73 years old (mean ± *SD*, 59.0 ± 10.0 years). Each subject was admitted to the CRC for a 10 h period of blood withdrawal, which started at 09.00 h. Blood samples were collected as described above every 30 min between 09.00 h and 18.00 h. Saliva samples were collected simultaneously by using Salivette tubes (Sarstedt, Newton, NC). At 11.00 h subjects ingested a gelatin capsule containing 0.3 mg melatonin (Nestle, Lausanne, Switzerland) with microcrystalline cellulose "Avicel" (FMC Co., Philadelphia, PA).

In both studies blood samples were stored at room temperature for 30 min during which they were protected from light, and then refrigerated at –5°C until sera were separated by centrifugation. Serum samples were then frozen at –20°C until assayed for melatonin concentration. Saliva samples were refrigerated at 5°C until centrifuged, and the filtrate frozen at –20°C until assayed for melatonin.

Melatonin concentrations were measured in 0.2 ml aliquots of serum samples collected between 21.00 h to 07.00 h; in 0.5 ml aliquots from serum samples collected before 21.00 h and after 07.00 h; and in 0.5 or 1 ml saliva samples, using a radioimmunoassay (RIA) kit (Buehlmann Laboratories, Allschwil, Switzerland) that employs the Kennaway G280 antibody. The limit of sensitivity of the melatonin assay is 0.5 pg/ml. Intra-assay coefficients of variation for control samples were 6.3% at 9 pg/ml and 6.1% at 22 pg/ml; the corresponding inter-assay coefficients of variation were 9.7% and 12.7% respectively.

The parameters of interest in study 1 were the time of "onset" and "offset" of the nocturnal increases in melatonin secretion; the area under the time-melatonin concentration curve (AUC) within the 25 h period of blood withdrawal; and the peak nighttime and the mean daytime melatonin levels. The onset and the offset of melatonin production were defined as the time points at which evening or morning serum melatonin reached a concentration 2 *SD* above the mean daytime level, as measured between 11.00 h and 17.00 h. In study 2 the parameters of interest were the AUC, measured between the time of melatonin ingestion and the end of the experiment (from 11.00 h to 18.00 h); the peak melatonin levels in serum and saliva; the acrophase of both serum and saliva melatonin profiles; the duration of a substantial increase in serum melatonin levels (above 20 pg/ml), and the duration of the increase in serum and saliva melatonin levels above 5 pg/ml (maximum daytime melatonin level we observed in our subjects) after the treatment.

Statistical analysis was performed using SAS v. 6.12® (SAS Institute, Cary, NC). Continuous variables were tested for normality by using PROC UNIVARIATE. Means of continuous variables in two groups were compared with unpaired *t* tests, unless indicated otherwise, and variances with *F* test (PROC TTEST). When the continuous variables were not normally distributed, we used Wilcoxon test (PROC NPAR1WAY). Two-way analysis of variance (ANOVA) was used to analyze the age by gender effects (PROC GLM). The Pearson coefficient of correlation was calculated to determine the relation between continuous variables. Two-by-two tables were analyzed by using Fisher's exact test (PROC FREQ). A p value < .05 was considered significant for all the tests. Mean values (± *SD*) are presented in the Results, unless otherwise specified.

RESULTS

The 25 h profiles of endogenous serum melatonin levels (mean ± *SEM*) observed in the groups of younger and older adult volunteers who participated in study 1 are presented in Figure 1 and Figure 2, respectively. The evening onset of melatonin secretion occurred significantly later ($p < .0001$) in the younger group (mean ± *SD*: 22.3 ± 1.06 h) than in the older group (20.8 ± 0.8 h); the offset of melatonin secretion in the morning also occurred significantly later ($p < .0001$) in the group of younger volunteers (9.1 ± 1.07 h) than in the older group (7.1 ± 1.09 h). The time of onset of melatonin secretion significantly correlated ($r = .95$) with the subject's habitual bedtime, which was 23.8 ± 1.06 h for

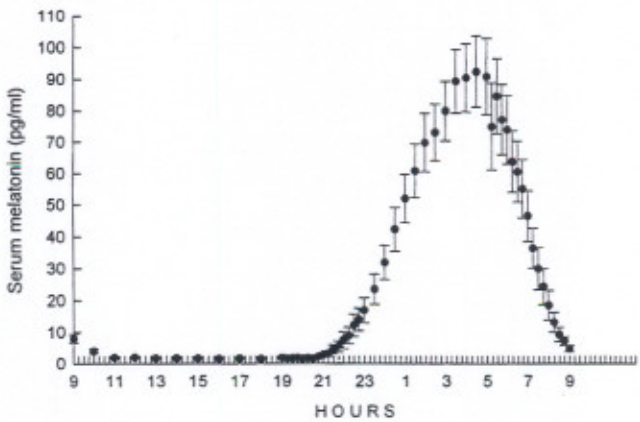

Figure 1. Profile of endogenous serum melatonin concentration in the group of younger volunteers (mean ± *SEM*; $n = 18$). Serum samples collected during a 25-h period.

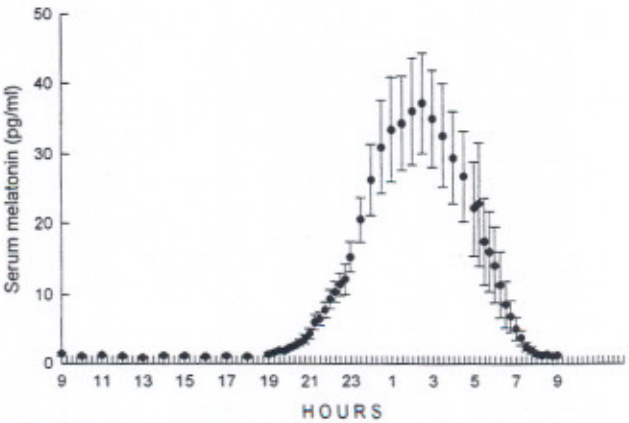

Figure 2. Profile of endogenous serum melatonin concentration in the group of older volunteers (mean ± *SEM*; $n = 18$). Serum samples collected during a 25-h period.

the younger group and 22 ± 0.88 h for the older group. The endogenous melatonin levels reached peak concentrations at 2.4 ± 0.86 h (mean ± *SD*) in the older group, and significantly ($p < .0001$) later, at 3.9 ± 0.93 h, in the younger group.

The peak endogenous melatonin levels were significantly higher in the younger group of volunteers (Wilcoxon test, $p < .0005$; Figure 3). One of the older subjects exhibited a peak melatonin concentration more than 3 *SD* higher than the other subjects in this age group and her peak value was considered to be an outlier. When this subject's data were excluded from the analysis, the comparison of variances in peak serum concentrations of the two age groups showed a significantly higher ($p < .0001$) variance among the younger subjects (100.9 ± 48.6 pg/ml) than among the older subjects (34.5 ± 15.4 pg/ml). Peak endogenous melatonin levels did not exceed 200 pg/ml in any of the subjects studied. There was no significant correlation between age and peak endogenous melatonin levels within either age group. Gender did not correlate with the AUC or peak endogenous melatonin levels in either of the age groups studied. The duration of the nocturnal increase in melatonin secretion (mean ± *SD*: 10.9 ± 1.2 h in the younger group, and 10.8 ± 1.3 h in the older group) did not correlate with age nor with the peak nocturnal melatonin levels. Mean serum melatonin AUC (± *SD*) in the younger group (596.3 ± 280.02 pg/ml h) was significantly ($p < .0001$) greater than that in the older group (246.9 ± 205.32 pg/ml h).

Similar differences in endogenous melatonin levels were observed in those subjects who participated in study 2: peak melatonin levels were again significantly lower in the older subjects ($p < .01$). Oral administration of a 0.3 mg dose of melatonin significantly increased circulating melatonin levels after 30 min ($p < .001$). Serum and salivary melatonin measurements showed that mean group AUC (± *SD*) for the younger group was 441.9 ± 121.07 pg/ml h (serum) and 136.4 ± 28.11 pg/ml h (saliva) (Figure 4). Mean group AUC (± *SD*) for the older group was 595.8 ± 312.09 pg/ml h (serum) and 165.5 ± 99.13 pg/ml h (saliva) (Figure 5). These parameters did not show a significant difference between the two age groups. There was, however, a high correlation ($r = .97$) between peak concentrations of melatonin in serum and in saliva (Table 1). Endogenous daytime melatonin levels (at 11:00 h) were similar in both age groups and did not exceed 5 pg/ml in serum or in saliva. Mean peak serum melatonin level following melatonin administration was higher among the older volunteers (254.9 ± 145.7 pg/ml) than in the younger group (170.2 ± 22.0 pg/ml); however, this difference did not attain statistical significance ($p = .10$). It might be explained by a significantly higher variance of serum melatonin levels (Figure 6) after treatment among the older subjects (76–486 pg/ml vs 142–205 pg/ml in the younger group; $p < .0001$). If we were to arbitrarily consider a 200 pg/ml serum melatonin concentration a maximum physiologic concentration for adults, more of the subjects in the older group (6 of 10) exceeded this level after melatonin administration than in the younger group (1 of 10; $p = .057$). The limited sample size did not allow us to confirm an effect of gender on the exogenously induced melatonin levels within each age group.

After administration of a 0.3 mg dose of melatonin it took 48 ± 4.9 min for serum melatonin levels to reach its peak values in the younger group, and 45 ± 6.7 min in the

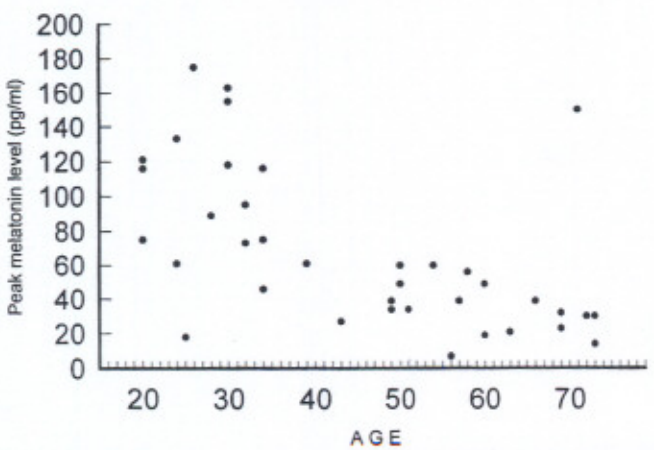

Figure 3. Individual peak endogenous serum melatonin levels in people of different ages ($n = 36$).

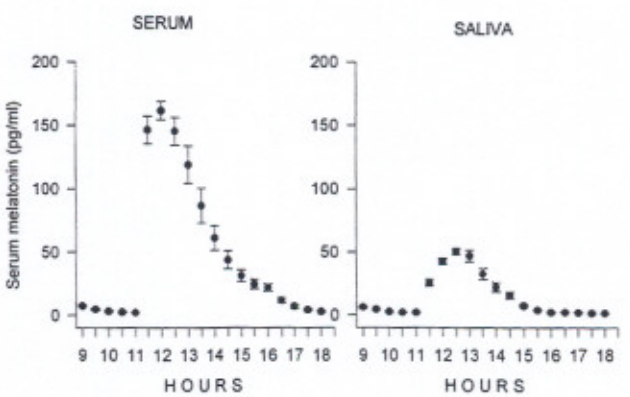

Figure 4. Profiles of serum and saliva melatonin concentrations in the group of younger volunteers (mean ± *SEM*; $n = 10$). Samples collected prior to and after the ingestion of a 0.3 mg dose of melatonin at 11.00 h.

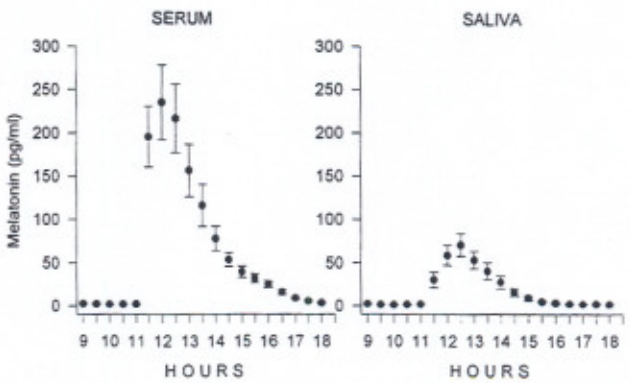

Figure 5. Profiles of serum and saliva melatonin concentrations in the group of older volunteers (mean ± *SEM*; $n = 10$). Samples collected prior to and after the ingestion of a 0.3 mg dose of melatonin at 11.00 h.

Table 1. Endogenous Melatonin Levels (nocturnal peak and at 11:00 a.m.) and Melatonin Levels in Serum and Saliva in Younger and Older Subjects After Administration of a 0.3 mg Dose at 11:00 a.m. (pg/ml)

		Endogenous			Following Treatment			
		Peak	At 11:00 a.m.		Peak		Acrophase (h)	
Gender	Age	Serum	Serum	Saliva	Serum	Saliva	Serum	Saliva
M	20	121	3.3	2.8	179	59	12	12.5
M	25	18	1.8	1.5	153	67	12.5	13
M	28	89	3.1	2.6	199	58	11.5	12
M	30	155	0.9	1.2	142	53	12.5	13
M	30	118	1.7	1.9	147	41	11.5	12.5
M	32	95	2.7	2.2	185	61	11.5	12.5
F	20	75	2.9	3.1	176	54	12	13
F	20	116	0.8	0.7	152	49	12	12.5
F	24	61	1.6	1.8	164	56	11.5	13
F	30	163	1.3	1.8	205	63	12	13
Mean	25.9	101.1	2.01	1.96	170.2	56.1	11.9	12.7
SD	4.7	43.5	0.9	0.7	22.0	7.4	0.4	0.3
M	49	34	2.4	1.6	87	14	11.5	12
M	50	60	1.1	1.4	286	95	12	12.5
M	57	39	0.9	0.7	179	46	11.5	12.5
M	73	30	3.2	2.6	89	27	11.5	12.5
F	49	39	2.6	1.8	354	93	12.5	12.5
F	50	49	0.8	1.4	326	101	11.5	12.5
F	58	56	1.6	1.3	243	73	12	12.5
F	60	23	2.8	2.1	76	29	11.5	12.5
F	71	150	1.9	2.3	486	134	12	12.5
F	73	14	1.9	2.4	423	132	11.5	12.5
Mean	59	49.4	1.92	1.76	254.9	74.4	11.75	12.45
SD	10.0	38.1	0.8	0.6	145.7	43.6	0.4	0.2

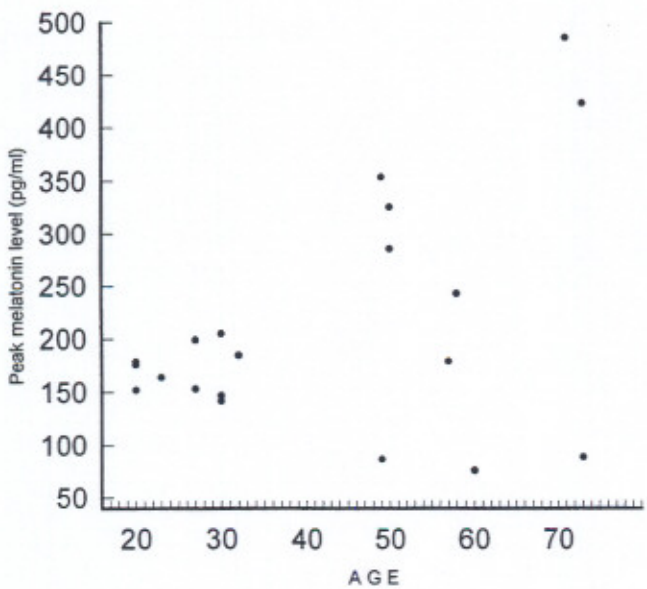

Figure 6. Peak serum melatonin levels in people of different ages after the ingestion of a 0.3 mg dose of melatonin at 11.00 h (n = 20).

older group (Table 1, acrophase); this difference between the age groups was not statistically significant. Salivary melatonin levels reached peak concentrations significantly later ($p < .001$) than serum melatonin levels: 102 ± 6.6 min after treatment in the younger group and 81 ± 6.4 min after treatment in the older group. Mean peak levels of salivary melatonin were not significantly different in the two age groups studied (74.4 ± 43.6 pg/ml in the older group vs 56.1 ± 7.4 pg/ml in the younger group); however, as with serum melatonin, variance was significantly higher in the older group ($p < .0001$). Individual peak levels of salivary melatonin were 26–34% of the corresponding peak serum levels in the younger group and 22–33% in the older group. The mean (± *SD*) group duration of a substantial increase in serum melatonin levels (> 20 pg/ml) after the treatment was not significantly different between two age groups: 5.2 ± 0.9 h for the older group and 4.5 ± 1.8 h for the younger group. The melatonin concentrations remained equal to or above the 5 pg/ml level (maximum daytime level without treatment) for 6.6 ± 0.24 hours (serum; mean ± *SD*) and 4.4 ± 0.24 h (saliva) in the older group, and 6.4 ± 0.21 h (serum) and 4.0 ± 0.43 h (saliva) in the younger group. These parameters did not differ significantly between the age groups. However, salivary melatonin levels declined significantly earlier ($p < .0001$) than serum melatonin levels in both groups.

Discussion

These data are consistent with earlier observations that mean peak circulating melatonin levels are depressed and generally less than 100 pg/ml in an older population (7–10). However, they also demonstrate that some individuals over 49 years of age do exhibit peak serum melatonin levels in

excess of 100 pg/ml (e.g., 150 pg/ml in a 71 year-old woman), and that some younger adults also may have peak circulating melatonin levels well under 100 pg/ml, similar to the values seen in most older people. The results of this study confirmed earlier observations (14–16) that after ingesting a 0.3 mg dose of melatonin younger adults tend to exhibit comparable levels of circulating melatonin (142–205 pg/ml), close to the reported physiologic peak levels for adults. However, this study also shows that older adults, after consuming the same low melatonin dose at daytime, show highly variable, and mainly supraphysiologic, peak melatonin levels (76–486 pg/ml).

Routine blood and urine analyses revealed no abnormalities in liver, kidney, or gastrointestinal functions in either our younger or older volunteers. The relatively low serum melatonin levels (e.g., 76 or 89 pg/ml) observed in some subjects after the administration of a 0.3 mg dose of melatonin might have resulted from a decrease in melatonin uptake from the gut or from a high first-pass hepatic extraction (18), whereas the very high levels observed in other subjects may have resulted from diminished catabolic enzyme activity.

The earlier onset of melatonin secretion in the older group of subjects in our study correlates with their earlier bedtime, reflecting the well documented advance in circadian phase of older individuals. Our data also suggested that there might be a gender difference in melatonin metabolism among older humans. The result was inconclusive due to the small number of subjects in each gender/age group, and to the unbalanced number of male and female subjects in each of the age groups. This issue will require further investigation.

At this time, the lack of an adequate body of clinical data precludes a reliable definition of "normal melatonin concentrations" and thus the clear identification of melatonin deficiency or excess. Both expressions imply not only certain "abnormal" levels of the circulating hormone but also clinical symptoms relatable to this abnormality. Our present knowledge of such clinical symptoms is limited. One possible manifestation of melatonin deficiency is insomnia which, reportedly, does correlate with lower melatonin levels in older populations (19). However, it is not established whether there is also a correlation between sleep quality and melatonin levels in younger adults. Low nocturnal melatonin levels have also been proposed as a "trait marker" for major depressive disorder (20), to be associated with a history of alcoholism (21), or with advanced stages of breast cancer (22). Reportedly, low circulating melatonin levels may also be a sign of a pineal tumor (23,24). Increases in circulating melatonin levels have been associated with hypothalamic amenorrhea (25,26), and with certain types of hypogonadism (27,28). Prolonged exercise in trained athletes may substantially increase melatonin levels (29), and may relate to the reproductive dysfunction that occurs in some athletes. Several reports suggest increased melatonin levels in the manic state (30). Many of these associations are tentative because the sample sizes involved in these studies were relatively small.

If exogenous melatonin is to be used in the future as hormone replacement therapy, similar to other hormones, the calculation of dosage will have to include consideration of age, gender, and individual differences in melatonin pharmacokinetics. If it is found to be important to obtain measurements of each individual's melatonin levels prior to treatment, then the development of simple sample collection and assay methods will be required. Although it would be ideal to be able to measure circulating concentrations of the hormone, saliva levels of melatonin, or urinary levels of melatonin's metabolite, 6-sulphatoxymelatonin may provide an adequate guide. Our studies and those of others (31,32) show that melatonin can be successfully measured in saliva. Peak saliva melatonin levels correspond to around 30% of peak serum levels. Daytime salivary levels of the hormone are surprisingly similar to those observed in blood (both measurements are well within the range of the assay sensitivity in the present study). It should be noted that increases in melatonin concentration after ingestion of the hormone can be detected significantly earlier in serum than in saliva, and salivary levels also appear to decline faster. These results suggest that salivary melatonin measurements may be used to estimate peak serum melatonin or a phase shift in melatonin production, but they may not be a reliable marker for the timing of the onset or offset of nocturnal melatonin secretion, as measured in blood.

In view of the diminished physiologic range in circulating melatonin levels in a group of people over 48 years of age, our data suggest that if melatonin therapy is to be initiated, there is a need for careful consideration of the dose to avoid the potential, and largely unstudied, untoward side effects of pharmacological levels of the hormone; e.g., body temperature suppression (33) or the increase in nocturnal prolactin levels (34).

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