Age, Alcoholism and Depression are Associated with Low Levels of Urinary Melatonin

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Two normal control populations, separated by 8,000 miles and 24 degrees of latitude, had similar six-month mean values for overnight urinary melatonin concentrations. These values were significantly higher than six-month values for depressed subjects and abstinent alcoholic subjects, while the means for the two clinical populations were similar. Age and urinary melatonin concentration in the control and clinical populations were inversely related, but the slopes of the linear regression equations were ten times steeper for the control populations than for the clinical populations. Differences in age and sex distributions accounted for some of the differences in values between controls and the clinical populations, although controls still differed from the clinical populations, even after sex and age were factored out. The disparate slopes for age and melatonin concentrations may contribute to some of the conflicting findings of studies comparing populations of different ages. The total melatonin content in the samples from alcoholic subjects, but not the depressed subjects, was lower than that for controls. The difference in the urinary melatonin concentration between the controls and the two patient groups was not accounted for by difference in duration of urine collection period, hours of sleep or body weight.

Key Words: melatonin, alcoholism, depression

INTRODUCTION

Melatonin production in humans has been used to index somatic, endogenous rhythmicity because of its regular periodicity, and to index noradrenergic activity because of its dependence on functional noradrenergic transduction (Checkley and Palazidou 1988). The rhythmic synthesis and release of melatonin from the pineal gland is controlled by a circadian pacemaker in the suprachiasmatic nucleus of the brain (Moore and Eichler 1972) which is phase-linked to the light/dark cycle by neural connections from the retina to the suprachiasmatic (Moore et al 1967). Signals from the suprachiasmatic reach the pineal gland through the superior cervical ganglion (Ariens-Kappers 1960). Norepinephrine, released from the sympathetic terminals at night, induces serotonin-N-acetyltransferase, the controlling enzyme in melatonin biosynthesis, leading to enhanced melatonin production.

A considerable number of reports have found that circadian rhythms are disturbed in depression (Miles and Philbrick 1988) and that the level of melatonin formation is lower than normal (Wetterberg et al 1979; Wirtz-Justice and Arendt

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Table 1

Mean duration of urine collection

	n	Hours Mean ± SE	Range (hours)
Controls			
St. Göran's	23	8.13 ± 0.19	6.2 to 10.2
UCLA	17	7.46 ± 0.24	5.8 to 8.9
Patients			
Depressed	11	8.93 ± 0.42	6.5 to 11.1
Alcoholic	19	6.99 ± 0.27	4.0 to 9.4

Values are the group means computed from the individual means for the time between the last nightly bladder emptying and the first voiding in the morning for each of the six monthly collection periods averaged. The range is the range of extremes of the mean values. The means were not significantly different. The populations were different ($F = 8.7$, $df = 3,66$, $p < 0.001$). Depressed subjects > St. Göran's controls = UCLA controls = alcoholic subjects, $p < 0.05$, Duncan's Multiple Range Test.

1979; Mendlewicz et al 1980; Beck-Friis et al 1984; Claustrat et al 1984; Nair et al 1984; Brown et al 1985; McIntyre et al 1986). This conclusion has been questioned, however, and the results have not been universally confirmed (Thompson et al 1988; Jimerson et al 1977; Stewart and Halbreich 1989).

Although there have been many studies of depression, there are only a few passing reports on melatonin production in subjects with alcoholism. One report suggests that alcoholism, like depression, may be marked by abnormal melatonin excretion (Wetterberg 1978). Another study found a complex relationship between alcoholism and melatonin production (Majumdar and Miles 1987), and a third found that ethanol disrupts the circadian melatonin cycle and elevates melatonin excretion (Murialdo et al 1991). Studies of alcohol and melatonin using animals are equally limited. Creighton and Rudeen (1988) found that acute ethanol administration delayed the nocturnal rise in pineal N-acetyltransferase, while Moss et al (1986) found low levels of pineal melatonin in rats given ethanol over a long period.

A sample of depressed and abstinent alcoholic subjects was included in a recent, large, worldwide multinational control study on normal seasonal changes in melatonin concentration. This provided the opportunity to examine the status of melatonin in multiple urine samples collected over an extended period of time from geographically dispersed clinical and control populations. We have taken this opportunity to compare nighttime urinary melatonin in these two clinical populations with two of the geographically separated groups of controls. Although most released melatonin is 6-hydroxylated in the liver and appears in the urine as 6-sulphatoxymelatonin and 6-hydroxymelatonin glucuronide, free melatonin was measured in this study to permit direct comparisons with urinary melatonin values in earlier studies and because 6-sulphatoxymelatonin reflects the enzymatic activities of at least two liver enzymes as well as melatonin concentration, and the sensitivity of these enzymes to environmental conditions has not yet been fully defined.

SUBJECTS AND METHODS

Urine Collection

The subjects were either outpatients or controls. The overnight urine sample was collected on the first Wednesday of each month (± one day) over a six-month period, from September 1988 to February 1989. The subjects were given graduated plastic beakers to measure the volume of their overnight urine and plastic vials to store the urine in a freezer. The specific instructions were to empty the bladder at bedtime (usually between 10:00 and 11:00) on the night before the collection, discard the urine, and record the specific time of the voiding. The total urine produced during the night, including the first morning urination (at approximately 07:00), was collected in a graduated plastic beaker. The total

Table 2

Mean duration of sleep

	Hours of sleep		Range	Extremes
	Hours	Mean ± SE	(hours)	(hours)
Controls				
St. Göran's	23	6.73 ± 0.18	4.9 to 7.8	4.0 to 9.5
UCLA	17	6.52 ± 0.21	4.7 to 7.4	3.0 to 9.0
Patients				
Depressed	11	6.86 ± 0.36	5.1 to 8.8	4.0 to 10.0
Alcoholic	19	6.54 ± 0.30	3.0 to 8.9	2.5 to 9.5

Values are the means of the mean sleeping time for each subject on the night of the six collection periods averaged. The range is the range of extremes of the mean values. The extremes are the range of the extreme individual values. The mean duration of sleep for the populations was not significantly different ($F = 0.3$, $df = 3,66$ $p = 0.03$).

volume of any nocturnal voiding was recorded, and a representative sample of the total urine was poured into a plastic bottle. The exact time of the morning voiding was recorded. The difference between the morning voiding and the evening voiding was the exact time during which urine was collected (shown in Table 1). The total hours of sleep were also recorded; the means for the populations are presented in Table 2.

The sample of overnight urine was taken to a collection point, where the bottles were frozen and stored at –20°C. Samples were not taken from subjects who had travelled more than 300 miles (500 km) north or south of their residence during the month preceding the sampling. At the end of the study, all urine samples from Los Angeles were transported to Stockholm on dry ice. All samples arrived frozen in Stockholm and were transferred to freezers kept at –20°C, where they were stored until assayed. Previous studies have shown that urinary melatonin is stable under these storage and transfer conditions (Wetterberg et al 1986). Previous studies have also found a strong correlation between urinary melatonin concentration (not content) in samples collected in this way and the 02:00 h peak value of serum melatonin ($n = 64$, $r = 0.8$, $p < 0.001$) (Almay et al 1987).

In addition, on each of the sampling days, a standard psychiatric examination, ratings of mood by two psychiatrists, and a self-rating mood scale were completed.

Subjects

Controls

The control subjects were part of a large multinational study on seasonal melatonin changes. Data from two reference populations were analyzed. The subjects were healthy university students and faculty members of either sex, aged 20 to 60, who volunteered for the study. They gave their written, informed consent to participate in the study. The subjects were given a brief medical and psychiatric examination to screen out those with somatic or mental disease or who were on medication. Oral contraceptives were permitted, provided they were used consistently throughout the study period. One reference population of 17 subjects (nine males and eight females, aged 26 to 58) was drawn from the faculty and students at the University of California at Los Angeles (UCLA). The secondary reference population of 24 subjects (14 males and ten females, aged 28 to 56) were staff members at St. Göran's Hospital, Karolinska Institute, in Stockholm. All of the subjects were Caucasians.

Depressed subjects

This sample consisted of four male and seven female depressed outpatients, all Caucasian, aged 23 to 65, who volunteered for the study, from the Serafen Outpatient Center in Stockholm, which is associated with St. Göran's Hospital. All subjects met the DSM-III-R criteria for major depression. Subjects were excluded if they missed more than two collection periods or used medications known to affect melatonin (such as beta blockers and monoamine oxidase A inhibitors). Three of the subjects were clinically depressed during the six-month collection period and were clinically rated as 1 on the three-point Comprehensive Psychopathological Rating Scale by two independent psychiatrists. Eight subjects were free of medication. Of the three clinically depressed subjects, one was on lithium and two were on antidepressants. The mean concentrations of urinary melatonin for the two subjects receiving antidepressants (0.148 and 0.090) were indistinguishable from the values for the eight subjects who were free of medication (mean = 0.148, range = 0.071 to 0.260). Subjects were asked to rate their degree of sadness on the two days preceding each monthly sampling, using the 100 mm Visual Analog Scale. Mean values for the group over the six-month collection period were 66 ± 10, 52 ± 8, 57 ± 3, 63 ± 10, 42 ± 13 and 60 ± 9 with a mean of 57 ± 4. The values for individual subjects over the course of the study ranged from 7 to 97.

Alcoholic subjects

This sample consisted of 19 alcoholic outpatients — nine Caucasian and nine Black males and one Caucasian female — in treatment at the West Los Angeles Veterans Administration Medical Center (Brentwood Division). The

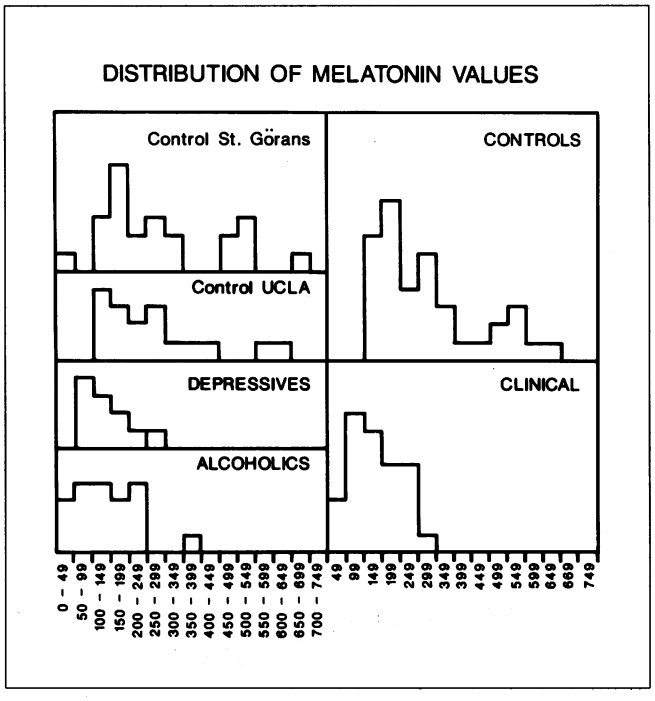

Fig. 1. Frequency distribution of urinary melatonin concentration. The Y axis presents the frequency of values of melatonin concentration within bins of size 0 pM to 49 pM along the X axis. The distribution for each population is presented separately on the left, and the distribution for combined control and clinical populations, on the right.

Table 3

Comparison of melatonin concentration and age of various diagnostic groups

Group	n	Melatonin (nM) Mean (± SE)	Age (years) Mean (± SD)
Controls	40	0.278 ± 0.026	38 ± 9
UCLA	17	0.273 ± 0.036	40 ± 10
St. Göran's	23	0.296 ± 0.038	36 ± 8
Male controls	23	0.214 ± 0.022	40 ± 9
UCLA	9	0.233 ± 0.022	44 ± 10
St. Göran's	14	0.208 ± 0.034	38 ± 8
Matched to alcoholic subjects	19	0.231 ± 0.020	37 ± 6
Female controls	17	0.364 ± 0.045	34 ± 7
UCLA	8	0.328 ± 0.064	35 ± 8
St. Göran's	9	0.395 ± 0.059	34 ± 6
Matched to depressed subjects	15	0.351 ± 0.096	38 ± 6
Depressed subjects	11	0.136 ± 0.018[acd]	48 ± 13[ac]
Female	7	0.138 ± 0.027[cd]	50 ± 3[ac]
Under age 61	9	0.144 ± 0.021[d]	44 ± 10
Alcoholic subjects	19	0.143 ± 0.019[abd]	54 ± 13[a]
Male	18	0.141 ± 0.020[bd]	54 ± 13[a]
Under age 61	11	0.140 ± 0.021[d]	45 ± 11

[a]differs from all controls; [b]differs from male controls; [c]differs from female controls; [d]differs from matched controls, $p < 0.05$
The groups were matched by deleting from the sex-matched patient samples subjects over age 61 and from the control populations, subjects under age 28. In addition, the male and female controls differ in age; the male and female controls at St. Göran's, but not at UCLA, also differ in melatonin concentration, $p < 0.05$. The depressed and alcoholic subjects did not differ significantly in melatonin concentration or age.

VA Medical Center is affiliated with UCLA. All the subjects gave their written informed consent to participate in the study. The subjects ranged in age from 29 to 73, and all met the DSM-III criteria for a lifetime diagnosis of alcohol abuse/dependence; none had a substance use disorder other than tobacco dependence. The subjects had abstained from alcohol between one month and more than ten years when they entered the study and remained abstinent throughout the six months of the study (determined by clinical monitoring). None of the subjects showed clinical evidence of any other major psychiatric disorder (DSM-III Axis I) during the study, and all denied having a history of psychiatric disorder other than alcoholism. Subjects were excluded if they suffered from a major medical condition (for example, renal or severe liver disease) which might interfere with melatonin formation or elimination, used medications known to affect melatonin, or missed more than two collection periods. One subject who took disulfram during the 12 months preceding the study was included. Values for the Caucasian and Black males were not statistically different when tested using the Student's t-test, and the values for the two groups were combined.

Analysis

Urine samples were analyzed for melatonin with a specific immunoassay developed for urine and blood (Wetterberg et al 1978). The assay had a sensitivity of 0.01 nmol/L. Inter-assay variability was 4.8% for melatonin levels above 0.15 nmol/L ($n = 60$). The melatonin values were expressed as both concentration (nM) and total amount in the collected sample (nmol).

Statistics

Group comparisons, for individual subjects and between subjects, were made using the BMDP 2V program, analysis of variance and covariance with repeated measures, using age and sex as covariates. Post-hoc comparisons between patient samples and subsets of age- and sex-matched controls were made using the Student's t-test. The data set for each individual consisted of four to six measured values, one for each month's sample. Since the statistical program used requires

a full data set (six values for each subject), missing values were supplied by interpolating between adjacent values. Interpolated values were used, rather than means, for missing values to account for possible time trends in the data. There were a total of 34 such adjustments (eight percent) within the 420 observation points. Analyses were conducted on both raw data and on logarithmic transformations of the melatonin values. Logarithmic transformations were used because urinary melatonin concentration is not normally distributed, while log concentration approximates a normal distribution. Log transformations generally produced higher levels of statistical significance than raw data, but the pattern of results was essentially the same. Least square linear regressions of age, sex and melatonin concentrations were also computed using BMDP 6d, bivariate scatter plots.

RESULTS

The two control groups had nearly identical mean urinary melatonin concentrations (F = 0.06; df = 1,37), differing by only eight percent (Table 3, Fig. 1), even though the populations were 8,000 miles and 24 degrees latitude apart. For both groups, age was inversely related to urinary melatonin concentrations (UCLA: r = –0.61; St. Göran's: r = –0.258). Moreover, the regression equations for both were also nearly identical (shown in Fig. 2) despite some differences in age and gender distribution within their populations. The women control subjects tended to be younger (34 ± 7 years) than the men (40 ± 9) (t = 2.3, p < 0.02) at both sites and had higher concentrations of urinary melatonin (0.378 versus 0.214 nM) (t = 3.5, p < 0.001) (see Table 3). gender differences in urinary melatonin concentration was found at both sites, but the difference was only statistically significant at St. Göran's (males 208 ± 34, females 418 ± 59 nM) (t = 3.3, p < 0.004) (see Table 3). The males and females at this location did not significantly differ in mean age (males = 38 ± 8; females = 34 ± 6 years); therefore, the melatonin difference could not be attributed to age.

The depressed and alcoholic groups also had similar values for mean urinary melatonin concentrations (F = 0.38, df = 27), which followed similar regression equations

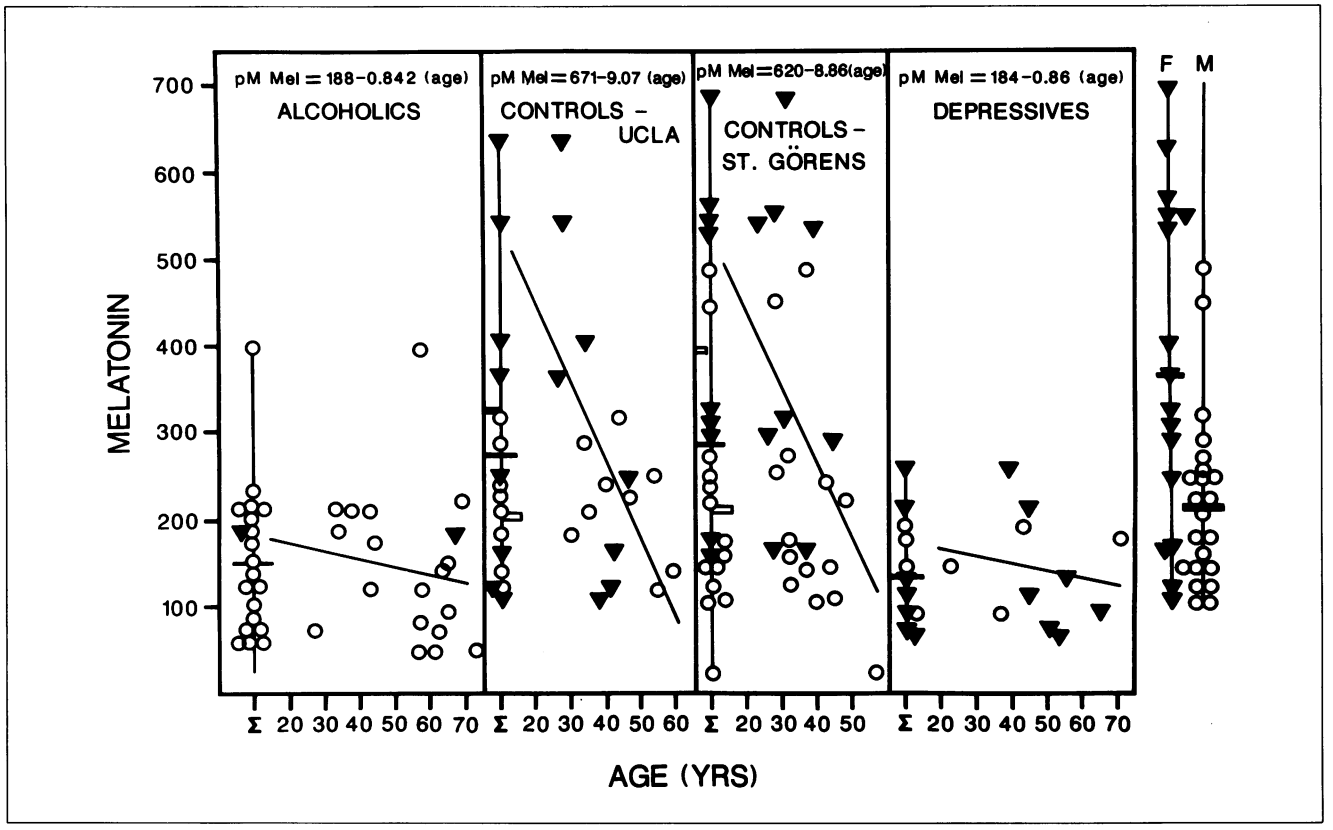

Fig. 2. Age and gender distribution of melatonin concentration in overnight urine from normal controls and patients with depression or alcoholism. Melatonin concentration in pM is on the ordinate and age on the abscissa. Open circles are the six-month mean of urinary melatonin concentration for males and closed triangles values for females. Values for each subject within a population are shown both on the line at 0, representing values for the total population, and at subjects' age. The population mean is represented by a full line intersecting the values at 0, while a half line on the right is the mean for males, and half lines on the left, the mean for females. The least squares regression line is presented above each column.

Table 4

Comparison of urine volume and total melatonin excretion by diagnostic group

Group	n	Urine volume (ml) Mean (± SE)	Melatonin content (nmol) Mean (± SD)
Controls (UCLA)	17	410 ± 49	107 ± 16
Controls (St. Göran's)	23	428 ± 39	108 ± 9
All controls	40	425 ± 29	105 ± 9
Male controls	22	439 ± 40	89 ± 9
Female controls	18	407 ± 42	125 ± 14[b]
UCLA			
Males	9	416 ± 68	90 ± 15
Females	8	402 ± 74	127 ± 25
St. Göran's			
Males	14	478 ± 57	95 ± 11
Females	9	355 ± 37	126 ± 14[b]
Depressed subjects	10	702 ± 148[ac]	92 ± 21
Alcoholic subjects	19	487 ± 63	67 ± 12[a]
Alcoholic subjects under age 61	11	498 ± 86	73 ± 15

[a]differs from all controls, $p < 0.05$; [b]differs from male controls, $p > 0.05$; [c]difference not significant when treated by ANOVA using age and sex as covariants. One patient on lithium was excluded.

relating melatonin and age (alcoholism: $r = -0.219$; depression: $r = -0.225$) (see Fig. 2). Both patient groups differed from the control groups in melatonin concentration (alcoholic subjects $F = 4.8$, $df = 1,55$, $p < 0.03$; depressed subjects $F = 8.6$, $df = 2,47$, $p < 0.005$) and in the slopes of the regression equations (see Fig. 2). The slopes for the clinical populations were much more shallow, signifying that age had less effect upon melatonin concentration. The patient and control populations also differed in the distribution of melatonin values (see Fig. 1). The melatonin values for the clinical population were unimodal, with a peak around 0.1 nM, while the values for controls were bimodally distributed with peaks around 0.2 nM and 0.6 nM. None of the values for the depressed subjects and only one for the alcoholic subjects reached the mean value for either set of controls (Fig. 2). The mean urinary melatonin concentrations were significantly lower for depressed and alcoholic subjects than for either group of control subjects, alone or together (see Table 3).

Age and gender correlated with urinary melatonin concentration in this study, so we examined their contributions to group differences in melatonin concentration. Urinary melatonin concentration for the depressed subjects remained significantly lower than that of the groups from UCLA, St. Göran's and the pooled controls after age and gender were included as covariants ($F = 5.9$, $df = 47$, $p < 0.005$). Comparing only those control and depressed subjects aged 60 or less (i.e., excluding two of the 11 depressed subjects over age 60) did not substantially change the mean melatonin concentration for the group (0.136 nM versus 0.144), nor did it alter the statistical significance of the difference from controls ($t = 2.5$, $df = 48$, $p < 0.02$). However, it did eliminate the statistically significant difference in age (38 ± 9 years for controls versus 43 ± 10 years for depressed subjects). The mean melatonin concentrations for the seven depressed female subjects were essentially the same as that for the entire sample of depressed subjects (0.136 versus 0.138 nM) and were significantly different from that of pooled female controls ($t = 3.2$, $df = 23$, $p < 0.004$).

A similar pattern was found for the subjects with alcoholism. Excluding the only female in the population and one male whose melatonin concentration was more than three standard deviations from the mean, an ANOVA using age as covariant indicated that age-corrected melatonin concentrations in urine from alcoholics was significantly lower than concentrations in urine from the male controls ($F = 4.3$, $df = 1,36$, $p < 0.04$). A comparison of all male controls and alcoholics within the same age range (under age 61) also showed the two groups differed significantly ($T = 2.9$, $df = 22$, $p < 0.01$).

The volumes of urine were the same for all groups except the depressed subjects (see Table 4). Six of the depressed subjects had morning volumes of urine that were consistently greater than the mean volume excreted by the controls. The one subject on lithium had volumes of urine ranging from 900 to 2,400 ml.

The melatonin content of the urine samples from the two control samples did not differ from each other or from the value for the depressed subjects (see Table 4). The melatonin content of urine from alcoholic subjects (67 ± 12 nmol) was significantly lower ($t = 2.45$, $p < 0.02$) than that for the

Table 5

Mean body weight (kg)

	Males		Females		Combined	
	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE
Controls						
St. Göran's	13	78 ± 3	11	64 ± 3[a]	24	72 ± 3
UCLA	8	78 ± 2	7	63 ± 4[a]	15	71 ± 3
Patients						
Depressed	4	67 ± 6	7	66 ± 5	11	66 ± 4
Alcoholic[c]	17	84 ± 4			17	84 ± 4[b]

[a]males > females for St. Göran's and UCLA populations; [b]combined depressed = UCLA = St. Göran's < alcoholic subjects, p = 0.05; [c]missing data for the female alcoholic subjects

controls (105 ± 9). The urinary melatonin content of all male controls (89 ± 9 nmol) was significantly lower than that for all female controls (125 ± 14 nmol, $t = 2.2$, $p < 0.03$), although the difference did not reach statistical significance at either site alone.

As shown in Table 2, while the hours of sleep did not differ between populations ($F = 0.3$, $df = 3.66$, $p = 0.3$), the collection time did (see Table 1) ($F = 8.7$, $df = 3.66$, $p < 0.01$; depressed subjects > than St. Göran's controls = UCLA controls = alcoholics at $p < 0.05$).

Table 5 shows that the males generally weighed more than females, that normal males weighed more than the normal females, that the alcoholic subjects weighed more than the others, and that the male and female depressed subjects did not differ in body weight. Body weight appeared to be unrelated to the population differences in urinary melatonin concentration.

DISCUSSION

By taking advantage of a much larger multinational, normative study of geographic seasonal rhythmicity, we were able to collect multiple urine samples evenly over many months. The resulting data is therefore more representative than that from single samples taken at some arbitrary time. The multinational natureof the normative study also allowed us to examine geographically separated populations so that the resulting data is also more geographically generalizable. However, the larger control study was concerned with naturalistic seasonal changes, and these advantages are paid for by variance in the times of urine collection and sleep, although the variance is constrained by work and hospital schedules. Thus, while rigid collection times could not be imposed in this study, variance was still relatively small. For the alcoholic outpatient population, for example, the mean time for beginning urine collection was 22:39, with a range over the six-month period of from 21:30 to 23:33 hours. The mean time for ending collection was 05:47 and the six-month range was somewhat larger, with extremes of 04:30 to 07:35, although most values were much closer to the mean. The design also prevented matching controls with the clinical populations for age and sex. Together, these factors and the small populations (11 depressed, 18 alcoholic and 41 control subjects) limit the generality of the results.

Nonetheless, the findings of this study are consistent with reports of a decline in melatonin production with age (Wetterberg 1979; Nair et al 1986) and with reports of low levels of melatonin production among depressed subjects (Wetterberg 1979; Wirtz-Justice and Arendt 1979; Mendlewicz et al 1980; Beck-Friis et al 1984; Claustrat et al 1984; Nair et al 1984; Brown et al 1985; McIntyre et al 1986) and alcoholic subjects (Wetterberg 1978). At the same time, these results are at variance with the reports of Jimerson et al (1977), Stewart and Halbreich (1989) and Thompson et al (1988) on depressed subjects and with the findings of Murialdo et al (1991) on alcoholic subjects.

Jimerson et al (1977) used a semi-quantitative bioassay based on the dermal bleaching of larval anuran skin in response to melatonin to quantify the 24-hour urinary melatonin rhythms of six normal and six depressed adults. They found little difference between the populations. Thompson et al (1988), using a radioimmunoassay for melatonin, failed to find a significant difference in the 24-hour plasma melatonin rhythm of nine sex- and age-matched pairs of normal control and depressed subjects who were drug-free for six weeks or more. Three of the depressed subjects, however, were candidates for a lobotomy and likely had a long complex drug history before the drug-free period. Stewart and Halbreich (1989) did not directly compare depressed and control subjects in their study of the effects of antidepressant treatment on melatonin in depressed patients, but they noted that daytime melatonin levels of the depressed patients were higher than reported values for normal subjects. Since melatonin concentrations are normally exceedingly low during the daytime, these researchers suggested their results indicated that their assay system was not specific. Murialdo et al (1991) found that alcohol markedly increased the daytime melatonin of their alcoholic patients between the ages of 35 and 54, and

slightly increased their nighttime melatonin; the increase in daytime and total urinary melatonin persisted over a two-week withdrawal period. These results likely reflect an acute response to alcohol. The alcoholics in our population, in contrast, were alcohol-free from one month to ten years before entering the study and remained abstinent throughout the study period.

Age and clinical status have been identified as factors which may complicate interpretation and account for disparate findings in depression (Thompson et al 1988). As is evident from slopes of the regression lines in Fig. 2 and confirmed by the results of the analysis of variance and covariance, using age and sex as covariates, age influences both urinary melatonin concentration and the magnitude of the difference between the two populations, although it does not account for the difference itself. However, it may contribute to the conflicting reports in the literature. The slope of the regression equations was ten times greater for controls than for the clinical populations (shown in Fig. 2). Accordingly, the young patients had melatonin values similar to those of the much older controls. This finding raises the possibility that both depression and alcoholism are associated with a premature aging of some components of the melatonin-generating system. The difference in slope also means that the magnitude of the population difference in melatonin concentration between control and either depressed or alcoholic subjects declines with age until, as algebraic solution of the regression equations shows, the differences become zero around ages 54 to 56. It is interesting to note that six of the nine pairs of subjects in the study by Thompson et al (1988) were in this age range. These data suggest that a detailed evaluation of the relationships between age and urinary melatonin concentrations in normal and patient populations is needed and may be more informative than simple cross-sectional comparisons.

While age may contribute to conflicts in the literature, differences between control and patient populations in this study are likely not the result of transient symptom expression since the data were collected over a six-month period. They are certainly unrelated to medication. All but three of the depressed subjects were drug-free for at least four months before the study and throughout the study period. All subjects in the alcoholic sample had abstained from alcohol for at least five months before entering the study and remained abstinent over the six-month collection period.

The samples were collected between September and February, when melatonin levels might be expected to be higher in the far Northern location of Sweden. The fact that urine melatonin levels were low in the depressed population in Sweden and the alcoholic population in California at this time of the year further supports the view that melatonin production is deficient in these clinical states. The similarity between the two geographically separate control groups and between the two geographically separate patient samples also suggests that reduced melatonin concentrations in the clinical samples were not likely the result of local environmental influences on melatonin production. However, as is the case for all comparisons between clinical and healthy populations, possible confounding effects of long-term debilitation, treatment, diet and secondary effects of disease cannot be ruled out.

The similar values for urinary melatonin concentration in the depressed and alcoholic populations could result from independent or common actions at some point of melatonin metabolism or physiology, perhaps at the level of sympathetic noradrenergic regulation of the pineal gland. To some extent, both clinical conditions are associated with corticotropic activity. Corticoids in both humans (Wetterberg 1983) and animals (Yuwiler 1985; Yuwiler 1989) blunt the pineal gland's response to stimulation which, in the animal studies at least, involved desensitization of noradrenergic receptors. The normal nocturnal rise in pineal melatonin was shown to be blunted in continuously intoxicated rats (Moss et al 1986) by a mechanism that also may involve reduced receptor function. A similar phenomenon may occur in humans.

Gender differences in serum melatonin in normal populations have not been found in other studies (Beck-Friis et al 1984). In this study, the gender difference in urinary melatonin was marked (90%) in the St. Göran's sample but smaller (47%) and not statistically significant in the UCLA sample (see Table 3). The values for the men at the two sites differed by 12% (0.208 versus 0.233), but the values for the women differed by a greater margin (20%; 0.328 versus 0.395). The data are insufficient to determine whether or not post-menopausal women tend to have higher values than men of the same age. This would be a valuable finding, because of the high proportion of post-menopausal women in many populations of depressed patients. The significant relationship between gender and melatonin in the Swedish sample could be simply chance but could also reflect gender-specific sensitivity to environmental differences between the two sites, such as day-length or temperature.

Individual sleep patterns (and associated urinary collection periods) were not disturbed in this study so that seasonal variations could be observed. Population differences in these variables were small. Mean sleeping time was statistically indistinguishable for the four populations, but the American populations slept 15 to 20 minutes less than their Swedish counterparts. This finding likely reflects adaptation to the long dark Swedish winter (latitude 59° N), compared with the more temperate winter in Los Angeles (34° N). The two Swedish populations also had longer urine collection periods than their American counterparts, and the collection time for the depressed subjects was longer than that for any other group. A longer collection period could lower melatonin concentration by diluting urine produced during the nighttime peak of melatonin release with urine of lower concentration. However, the ten percent longer collection time for the Swedish depressed patients compared with the Swedish

controls cannot account for the 60% difference in melatonin concentration.

Although population differences in body weight have been suggested to contribute to age-related changes in melatonin content in rats (Yie et al 1992) and humans (Ferrier et al 1982; Arendt et al 1982), there appeared to be no consistent relationship between body weight and population differences in melatonin in this study. The depressed subjects had the lowest body weight and the alcoholic subjects, the highest, although melatonin concentration was below normal for both groups.

Therefore, this findings of this study indicate that age, alcoholism and depression are associated with low nocturnal urinary melatonin concentration.

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