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ORIGINAL ARTICLE

Declining melatonin levels and older people. How old is old?

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Abstract

OBJECTIVES: The purpose of this study was to investigate whether melatonin levels in older cohorts within the 'aged' population were significantly lower than in younger 'aged' individuals and hence point to a possible confound in cross-sectional studies which group individuals over 55 in one category.
SETTING AND DESIGN: Melatonin levels of 35 North Queensland residents over 55 years of age living in an aged-care facility, a retirement village or the general community were compared across three age groups.
METHODS: Subjects were ten 56–65 year olds, eighteen 66–75 year olds and seven over-75 year olds. Information was obtained on sleep, awakening, medical conditions and medications, with subsequent exclusion of those with known medical conditions and/or medications. Melatonin was collected by salivary samples at 2200 hours and concentrations were determined by immunoassay.
RESULTS: Mean melatonin levels were significantly lower ($p=.03$) in the 'oldest' (over 75 yrs) group compared to the 'youngest' (56–65 yrs) group.
MAIN FINDINGS: The results of this preliminary study indicate that within the older population, melatonin levels appear to decline significantly with age.
CONCLUSIONS: Future studies of melatonin and ageing may benefit from a longitudinal approach, with older subjects sampled across time.

Abbreviations and Units:

mL	millilitres
L	litres
pmol/L	picomole/litre
pg/mL	picogram/millilitre
μL	microlitre
ANOVA	Analysis of Variance

Introduction

The pineal gland, and as a consequence, melatonin, attracted wide interest in ageing research when pineal glands from young mice were transplanted into old mice. It was reported [1] that as a result older mice lived up to a third longer and that "(P)ineal cross-transplantation provides clear cut evidence for the central role of the pineal gland in the initiation and progression of senescence" [2, p.456].

While circadian variation and melatonin disruption are well documented [3, 4, 5, 6, 7], life time changes in human melatonin production are yet to be definitively determined. Progressive changes in melatonin production and release have previously been implicated in the process of ageing [8, 9], and, consequently, sleep problems experienced by the elderly [10]. While such claims are now contentious, research has suggested that with age, melatonin production declines.

Among this research are reports that younger people (29.2 ± 6.5 yrs) when compared to older people (60 ± 8 yrs) demonstrate significantly higher peak endogenous melatonin concentrations [11], although the high inter-individual variability evidenced in this investigation warrants caution in interpretation. Broadly, younger people have been said to demonstrate greater inter-individual variability in melatonin concentration compared to older people, while more extreme claims include a 50% decline in melatonin by 40 years of age compared to that evident in youth [12].

More recent investigations have provided greater rigor in assessment of potential age related changes in melatonin. In these studies, younger and older subjects were shown to exhibited similar melatonin rhythms, while also evidencing similar concentrations of 24 hour melatonin; nocturnal peaks of melatonin were comparable [13]. An extension of this research [14] examined the timing of sleep-wake episodes and the timing of the rhythm of plasma melatonin secretion and concluded that older people display a difference in the timing between the two. The results did not lend support to a role for melatonin in sleep disruption in older people, but rather suggest an altered phase relationship between the timing of sleep-wake episodes and the timing of the rhythm of plasma melatonin. The greater than tenfold inter-individual variability in reported melatonin concentrations is, however, noteworthy. Given that there were 15 older subjects and 33 younger subjects included in this investigation, the lower means, standard deviations and range of melatonin concentrations for the older group may warrant further exploration.

Other research has demonstrated that the mean timing of onset and offset of melatonin secretion were similar in older and younger subjects and that no significant age differences were apparent in amount of endogenous melatonin [15]. The investigators however urged caution in interpreting these findings, as low subject numbers and age of the oldest participant limited the power of analysis. It is further noteworthy that one older male subject with very high melatonin is a statistical outlier, thus substantially influencing the mean outcome comparisons made between younger and older subjects. Results of statistical analysis of the raw data (by ANOVA) with inclusion of the outlier, $F(1,21) = 0.557$, $p = 0.64$, compared to results that exclude the outlier, $F(1,20) = 3.163$, $p = 0.091$, produce enough difference to further warrant caution in interpretation of the findings (particularly given the low subject numbers noted).

Other researchers have examined the role of melatonin in ageing through melatonin administration trials. Results from this research are also mixed, with some reports [16] suggesting that melatonin administration may be beneficial in advanced age while others suggest that it is not [17]. A review of the literature relating to melatonin administration in treating insomnia in the elderly [18] concluded that while further research is necessary, improvement may occur in subjects that have documented low melatonin levels during sleep and in those who chronically use benzodiazepines.

Most researchers categorise subjects as young or old. Such comparisons are informative and necessary, but these categories can also become somewhat arbitrary. Given the poor understanding of the function defining melatonin levels with ageing, it may be the case that differences exist within 'older' cohorts. A clearer delineation between 'older' subjects according to their age may allow for further insight into any differences that occur within the cohort. Such differences may be masked by grouping subjects as 65+, and classifying them as 'elderly'.

In order to explore this possibility, an exploratory study was undertaken. The aim of the current investigation was to compare salivary melatonin levels in an older cohort, using clearly bracketed age groups to explore any within-group variation potentially related to age.

Methodology

Subjects: Subjects were drawn from two areas: residents in a retirement village in north Queensland (n=28), and retired people living in the community (n=22) in North Queensland. The majority of the subjects were members of the University of the Third Age (U3A), an organisation that (locally) actively participates in various research projects. Informed consent was obtained from all subjects and the project was overseen by the JCU ethics committee. From the original sample of 50 subjects, 37 subjects were able to successfully complete the experimental protocol. They were medication free. One 71 year old subject failed to provide a sample, and was excluded. Thus, the final sample comprised ten 56–65 year olds, eighteen 66–75 year olds, and seven 76–84 year olds.

Apparatus: A sleep log and general questionnaire were utilised. All subjects residing outside of the aged care facility received a freezer pack that contained test tubes, an instruction sheet, and a separate time sheet.

Procedure: All subjects received the collection kits on the day of sampling. A questionnaire related to sleep, awakening, medical conditions and medication was completed. Those with known medical conditions, and those taking medication were excluded and invited to participate in an alternative study. All participants were trained in saliva collection. Participants provided a saliva samples at 2200 hrs that evening. Actual collection times differed slightly between participants. A one-way ANOVA, however, indicated no significant

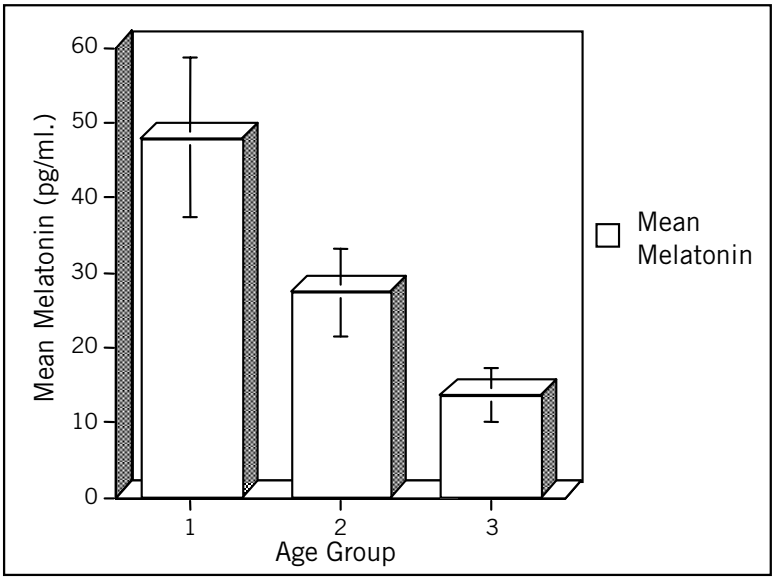

Figure 1: Mean melatonin levels across the three age groups (showing standard error)

time variation for collection between age categories, $F(2,32) = 2.378$, $p = 0.109$.

At least 4ml of unstimulated saliva was collected in two 10ml disposable centrifuge tubes. Subjects made their own collections and were asked to immediately place the samples in a freezer in the pre-prepared collection pack. The specimens and time sheets were collected the following day. All participants indicated that they had followed the protocol provided (with non-significant time variations as indicated above). After collection, samples were stored at –20°C and later thawed at 4C.

Melatonin RIA Determination

Salivary samples were not labelled according to age category and were assayed by an independent biochemist at La Trobe University, Victoria.

The method used was similar to that described in a previous validation and comparison of salivary melatonin to plasma melatonin [19]. It was based on 125I-labelled 2-iodomelatonin as tracer (specific radioactivity 81.4 TBq/mmol; New England Nuclear, USA), used at 3 pmol/L in the assay reaction mixture, and a highly specific antiserum (code G280; Vaughan, 1993) at a dilution of 1:2.25 x 106 to give 55% B0 maximum tracer binding. The sampled volume was 150 μL, and the final reaction volume was 750 μL. Standard solutions (0–800 pmol/L; 0–180 pg/mL) were prepared in advance in melatonin-free saliva (obtained by treatment of pooled saliva samples with charcoal and C18), by dilution from a concentrated stock solution in ethanol, and aliquots were stored at –20°C, ready for assay. A solid phase second antibody reagent (100 μL Sac-Cel; Immuno Diagnostics Limited, UK), washed and resuspended pH 7.4 diluent buffer, was used to separate antibody-bound and free melatonin.

Assay detection limits 'ALD' and 'FLD' averaged 2.3 and 5.3 pmol/L (0.5 and 1.2 pg/mL), respectively, using the method outlined, and the mean upper limit of the reliable working range was recorded as 740 pmol/L (170 pg/mL). Low-melatonin saliva samples tested in all assays of the current series (n=8) showed between-assay variability to be as high as 15.7%cv at 20.5 pmol/L and 20.3%cv at 6.5 pmol/L, concentrations close to the lower end of the assay's working range. Estimates of between-assay variability were less than 12%cv for saliva melatonin concentrations higher than 40 pmol/L.

Results

A one-way ANOVA indicated that there were no significant gender differences in melatonin concentrations, $F(1,33) = 0.579$, $p = 0.452$. Thus, male and female data for each age category were combined.

A further one way ANOVA indicated between group differences, $F(2,32) = 3.940$, $p = 0.030$. Tukey post hoc comparisons indicated that the youngest and oldest age categories differed significantly (mean difference = 34.3157, SE. = 12.6711, $p = 0.028$, see Figure 1). No other significant differences were detected. Raw data appear in the Appendix.

Discussion

The current findings indicate some support for progressively lower melatonin concentrations in older subjects with increasing age. While studies between young and elderly subjects are valuable, comparisons within an older cohort may also be necessary. Specifically, when investigating potential age related shifts in melatonin, further delineated aged categories may show differences that can potentially be masked by group analysis. This exploratory study found a significant difference in melatonin concentration between the oldest elderly group (mean age = 80 yrs) and the youngest elderly group (mean age = 60.6 yrs). The intermediate elderly group (mean age = 69.44 yrs), while not differing significantly from the other two

groups, showed lower concentrations of melatonin than the youngest group and higher concentrations than the oldest group.These results are suggestive of progressive declines in endogenous melatonin with increasing age.

There are however obvious limitations in this investigation that render conclusions difficult. The technique adopted in the current study relied on salivary sampling of melatonin, and sample collection at 2200 hours only. While this is when the nocturnal rise in circulating melatonin is evident, clearly 24-hour sampling (or at least peak time sampling) is desirable. Further, subjects were not kept in controlled environments, but rather samples were collected in the usual home environment. It is nevertheless extremely unlikely that the observed differences occurred by chance.

Subject numbers within each cohort also render any interpretation of differences according to cohort somewhat tenuous. The oldest group contained only seven subjects. While this is comparable to previous studies in the area, future investigations should aim to increase group sizes considerably. Ideally, subjects should also be sampled regularly over time to provide a within subject design in order to track any progressive changes in melatonin due to ageing. A within-subjects design would also minimise inter-individual variability evident in previous well controlled investigations [14, 15].

Despite the limitations of the current investigation, the statistically robust significant differences noted within the oldest elderly cohort when compared to the youngest elderly cohort are of interest. The findings suggest that clearer categorisation of subjects according to age may be of benefit. Longitudinal investigations incorporating tighter controls and a larger sample of the ageing population appear desirable.

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Appendix: Raw data for each age group

Age 1	Melatonin pg/ml	Age 2	Melatonin pg/ml	Age 3	Melatonin pg/ml
63	66.3	72	49.9	82.0	9.1
61	74.8	68	5.1	78.0	6.2
64	55.9	68	4.0	80.0	7.6
57	19.9	74	11.9	81.0	8.6
57	8.9	67	74.2	84.0	15.1
59	97.2	75	23.0	79.0	14.6
60	8.5	75	22.4	76.0	34.1
63	20.4	69	7.1		
61	89.9	66	23.9		
64	37.5	71	54.5		
		67	5.3		
		69	20.8		
		70	6.9		
		(71)	.		
		68	51.2		
		69	83.9		
		67	29.0		
		68	14.3		
		67	5.4		
Mean	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
60.6	47.93	69.44	27.38	80	13.61
	SD		**SD**		**SD.**
	33.4		24.92		9.66