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Qualitative and quantitative changes of melatonin levels in physiological and pathological aging and in centenarians

Abstract: Melatonin secretion is an endogenous synchronizer, and it may possess some anti-aging properties. Thus we examined melatonin levels in physiological aging, in extreme senescence and in senile dementia. In healthy old (age 66–94 yr) and young subjects (age 23–39 yr) and in demented patients (age 68–91 yr) plasma melatonin was measured by radioimmunoassay in eight serial blood samples. In centenarians (age 100–107 yr) melatonin levels were estimated by assaying urinary 6-hydroxymelatonin sulfate (aMT6s) in two different urine samples collected from 08:00 to 20:00 hours and from 20:00 to 08:00 hours. These data were compared with the aMT6s excretion of old and young controls. Elderly subjects, demented or not, exhibited a flattened circadian profile of plasma melatonin, because of the suppression of the nocturnal peak. An age-related decline of the circadian amplitude of the melatonin rhythm occurred in old subjects, especially in demented individuals. Furthermore, the melatonin nocturnal peak was significantly correlated with the severity of the cognitive impairment. aMT6s urinary excretion also declined with age. However, as in young controls, in centenarians the aMT6s excretion was significantly higher at night than during the day. In conclusion, pineal melatonin secretion is affected by age and by the degree of cognitive impairment. In centenarians the maintenance of the circadian organization of melatonin secretion may suggest that the amplitude of the nocturnal peak and/or the persistence of a prevalent nocturnal secretion may be an important marker of biological age and of health status.

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Introduction

Blood melatonin concentrations, primarily derived from the pineal gland, exhibit evident circadian fluctuations with the highest levels during nighttime and the lowest values during daytime [1]. Melatonin circadian rhythm is modulated by the hypothalamic suprachiasmatic nucleus (SCN), i.e. the biological clock [2, 3]. The SCN receives light impulses throughout the retinohypothalamic pathway and it is related to the pineal gland via the peripheral sympathetic nervous system. Although light inhibits melatonin secretion, it is important to note that the melatonin circadian rhythm has an endogenous origin. Furthermore, melatonin can directly influence the SCN throughout complex feed forward and feed back mechanisms [4, 5].

Noradrenalin (NA) after its binding to $\alpha 1$ and $\beta 1$ adrenergic receptors, is the main neurotransmitter involved in melatonin secretion. During light exposure the NA release is inhibited after the hyperpolarization of the retinal photoreceptor cells [6]; on the contrary, the darkness leads to the activation of the system and the melatonin secretion increases [7]. Melatonin is then rapidly metabolized, especially by the liver, and excreted in the urine. The urinary levels of 6-sulfatoxymelatonin (aMT6s), the main melatonin urinary metabolite, closely parallel plasma melatonin concentrations [8].

The melatonin circadian rhythm becomes apparent after the second or third month of life, reaching the highest nocturnal levels in children aged 1–3 yr. Thereafter, the melatonin circadian fluctuations, although persisting, show two significant declines: the first during puberty and the second during the progression from adulthood to senescence [9–12]. The actual nocturnal melatonin decline is 40–50% in elderly subjects [13]. Thus while aging may be considered a condition of relative melatonin deficiency, it seems unlikely that aging is merely a consequence of the loss of melatonin as provided by Rozenzweig et al. [14].

Melatonin has several properties that could be considered as anti-aging. The first and well-studied effect of melatonin concerns its positive action on the duration and quality of sleep and on the speed of falling asleep [15]. This is particularly interesting because sleep disturbances or changes in the sleep–wake pattern are often observed in geriatric population. This hypnotic effect of melatonin seems to be mediated by reducing body temperature [16].

Many groups have studied the role of melatonin as free radical scavenger [17], showing that melatonin protects against the oxidative damage [18] both directly and

throughout the enhancement of other antioxidant enzymes [19–25]. These effects, well evident at pharmacological melatonin concentrations and during challenges that cause a relevant number of free radicals, are also evident at physiological melatonin concentrations [26]. In addition immunoenhancing effects and protective activity from cytotoxic-mediated apoptosis have been described for melatonin [27, 28]. Finally, there is evidence that melatonin limits the stress-related corticotropin response [5] and the growth of spontaneous and induced tumors [29].

The persistence of a good melatonin signal, i.e. of a good amplitude of melatonin circadian rhythm, is significantly related to the stability of the circadian system [30]. Beside a general age-related reduction in melatonin concentration, the amplitude of nocturnal melatonin secretion shows a great inter-individual variability, being, accordingly to many evidences, genetically determined [31].

Taken together these data suggest that changes of the melatonin circadian rhythmicity play a role in some age-associated brain degenerative changes, already present in physiological aging and amplified in age-related diseases such as Alzheimer's dementia or cardiovascular pathologies. However, only few data are present in the literature about melatonin circadian rhythm in long-living subjects or in centenarians, probably the better example of selection because of genetic and environmental factors. The aim of this work was to study the circadian organization of melatonin secretion in physiological aging, in some pathological age-related conditions such as senile dementia, and in highly selected population of centenarians, in order to determine some qualitative and quantitative secretory changes and to search for potential relationships between age or age-related diseases and the same changes.

Subjects and methods

Protocol A

In this study, 33 old healthy subjects, aged 66–94 yr (mean age = 81.7 ± 6.5 S.D.), hospitalized for minor somatic diseases and without alterations of consciousness or of sleep–wake cycle, and 38 old demented patients, aged 68–91 yr (mean age 81.0 ± 5.9 S.D.), including subjects with Alzheimer's disease (AD) and with vascular dementia (VD) were studied. The diagnosis of senile dementia was performed according to the criteria of DSM-IV for primary degenerative form and of NINCDS-ADRA for probable AD [32], while CT scan images and the criteria of the Hachinsky ischemic score was followed for VD [33]. The severity of dementia and the degree of cognitive impairment were assessed by the Clinical Dementia Rating (CDR) and the Mini Mental State Examination (MMSE), respectively.

Thirteen young healthy subjects, aged 23–39 yr (mean age 36.9 ± 4.3 S.D.) were chosen as controls.

For both young and old subjects the presence of major depression (DSM-IV) or of minor depressive symptoms (Hamilton Rating Scale on Geriatric Depression Scale) was considered as exclusion criteria.

Subjects were drug free from at least 2 weeks before the study, and the medical history and clinical or biochemical screening tests excluded alcoholism, endocrine, infectious or neoplastic diseases and hepatic or renal failure.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethical Committee of the Department of Internal Medicine of the University of Pavia. Written informed consent was also obtained from all subjects or, when appropriate, from their legally appointed guardians.

The study was not carried out in a defined season but in different months of the year, and in every case at least 7 days after hospital admission in order to allow the subjects' synchronization to hospital schedule (meals at 08:00, 12:00 and 19:00 hours, and sleep in darkness from 21:00 to 07:00 hours).

The circadian rhythm of plasma melatonin was evaluated by serial blood samples collected every 4 hr during daytime (from 08:00 to 20:00 hours) and every 2 hr during nighttime (from 20:00 to 08:00 hours). In order to avoid venipuncture stress, an indwelling catheter was inserted into an antecubital vein before the study. The nighttime blood samples were collected under yellow light of 100–400 lux directed only on patient's arm; in our experience this is a good way to avoid interference with sleep and melatonin secretion. Each subject maintained a regular sleep schedule for a week before and during the study.

Plasma melatonin was determined by radioimmunoassay (Nichols Institute BV, Wychen, The Netherlands) with diethyl-ether extraction of 500 mL volume of anticoagulated plasma, and the results were expressed as pg/mL; the intra- and interassay coefficients of variation were below 10% and the low detection limit was 3 pg/mL.

Protocol B

Two investigators visited 20 centenarian subjects, free living or living in nursing homes (three males and 17 females, aged 100–107 yr, mean age = 101.5 ± 1.87 S.D.) and 22 old healthy subjects (seven males and 15 females, aged 71–93 yr, mean age 83.6 ± 6.09 S.D.), also either free living or living in a nursing home. In both groups of elderly subjects a careful geriatric multidimensional assessment was performed, as well as the clinical and biochemical screening test, and, as described in protocol A, the presence of relevant diseases was considered as exclusion criteria. Seventeen young healthy subjects (six males and 11 females, aged 22–35 yr, mean age 28.7 ± 3.34 S.D.) were considered as controls. The study was approved by the local Ethical Committee and each subject gave written informed consent.

Due to the technical and ethical limitations in studying centenarians, the circadian organization of melatonin secretion was evaluated by measuring urinary aMT6s, the main melatonin metabolite in two different urine samples collected between 08:00–20:00 hours (daytime) and from 20:00 to 08:00 (nighttime). The urine volumes and the creatinine clearance were lower in healthy old subjects and in centenarians, than in young controls, but in any case mean values were within the normal range.

Urine aMT6s determination was provided by high sensitive enzyme-linked immunosorbent assay procedure (Bühlman Laboratories AG, Allschwill, Switzerland). The assay was performed after urine sample dilution (1:200) with incubation buffer. The sensitivity was <0.35 ng/mL. The intra-assay precision ranged between 3.7 and 8%. The inter-assay precision was calculated from the results of four consecutive pairs of values obtained in 10 different runs and ranged from 7 to 10%.

Statistical analysis

Data are expressed as mean ± standard error and compared using the Mann–Whitney *U*-test; the relationships between hormonal values and clinical features were calculated by the Spearman Rank Order correlation. The threshold level of significance was $P < 0.05$. The statistical significance of the hormonal fluctuations throughout the 24 hr cycle was validated by the single and mean cosinor method [34] which allows for the calculation of the mean level, or mesor (M, a rhythm adjusted mean), the amplitude (A, one-half the difference between the peak and the nadir values of the circadian profile) and the crest-time or acrophase (Ø, the time of the peak level of the function), with the corresponding 95% confidence limits. The comparison of the rhythm parameters was carried out using the mesor test and the amplitude-acrophase Hotelling's test, according to Bingham et al. [35].

In order to determine the total melatonin secreted during a 24 hr cycle, the melatonin index was measured by calculating the area under the curve and using the lowest melatonin concentration as baseline [36].

Results

Fig. 1 summarizes the circadian pattern and parameters of plasma melatonin in healthy young and old subjects and in demented patients. The circadian profile of plasma melatonin was clearly flattened in old subjects, demented or not, when compared with young controls, because of a selective impairment of nocturnal melatonin secretion. Indeed, both the melatonin nocturnal peak and melatonin index were significantly lower in healthy old subjects and in demented patients than in young controls, with a significant negative relationship with age of the subjects ($r = -0.388$, $P < 0.001$ and $r = -0.401$, $P < 0.001$, respectively). Furthermore, the severity of cognitive impairment (MMSE score) was significantly linked to the nocturnal melatonin peak ($r = -0.340$, $P < 0.037$).

At the population mean cosinor analysis, the circadian rhythm of plasma melatonin reached statistical significance in the three groups evaluated, without differences in mesor values among the groups. However, a significant reduction of the circadian amplitude was found in old healthy subjects and especially in the demented ones (Table 1).

In centenarian subjects, pineal secretory activity showed a further age-related decline, as evidenced by the reduction of urinary aMT6s (Fig. 2A) both in diurnal and nocturnal urine samples and in the total excretion rate. A significant negative relationship linked the subjects' age to the day and night aMT6s ($r = -0.449$ and $r = -0.785$, $P < 0.001$, respectively). However, by expressing the diurnal and nocturnal aMT6s excretion as percent of the total 24 hr amount, the nocturnal values were significantly higher than the diurnal ones in both centenarians and young healthy

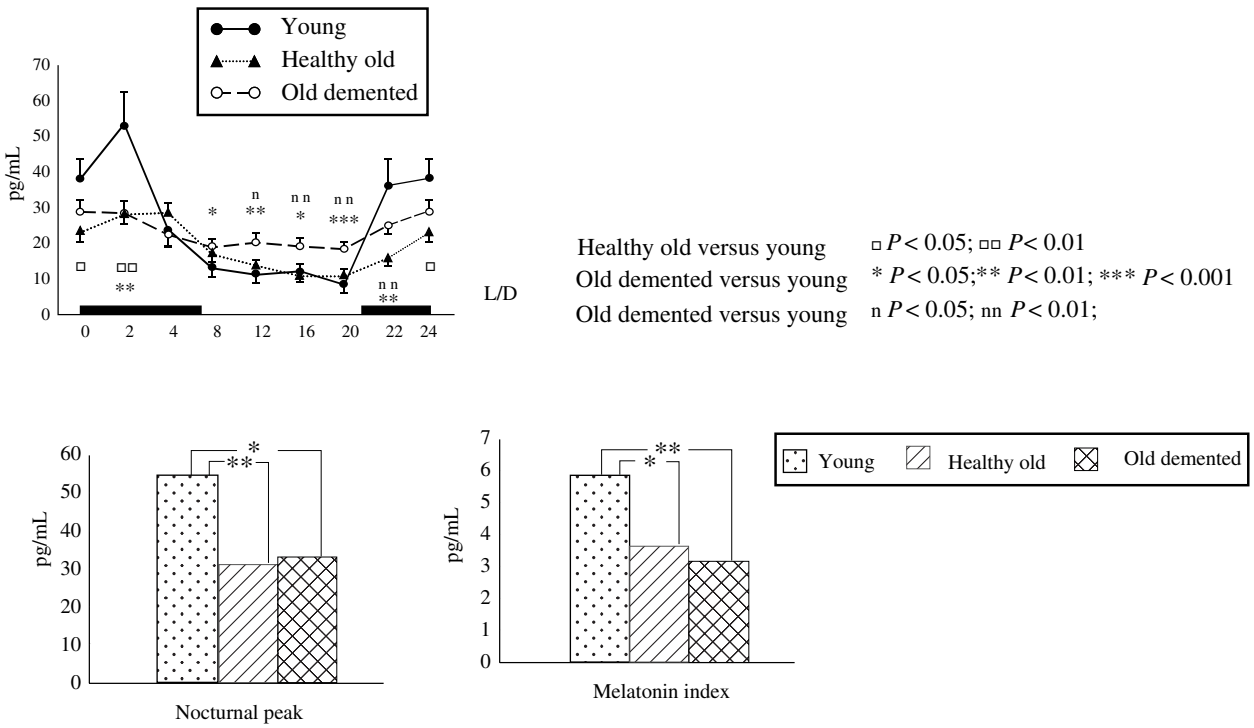

Fig. 1. Plasma melatonin circadian rhythm in healthy young and old subjects and in demented patients (mean ± S.E.).

Table 1. Population mean cosinor summary of melatonin circadian rhythm in the three groups of subjects

	P-value	Mesor M (mean ± S.E.M.)	Amplitude A (mean ± S.E.M.)	Acrophase hours (95% CL)[a]
Young subjects	0.0002	21.12 ± 3.23	17.16 ± 2.91	01:30 (00:16–03:08)
Old subjects	0.0001	16.23 ± 1.43	8.80 ± 1.10	03:32 (02:49–04:29)
Old demented	0.0003	19.34 ± 1.83	7.72 ± 1.43	01:53 (01:02–02:50)

[a]Phase reference: local midnight – 360° = 24 h; 15′ = 1 h.
Hotelling test: $*P < 0.01$; $**P < 0.001$.

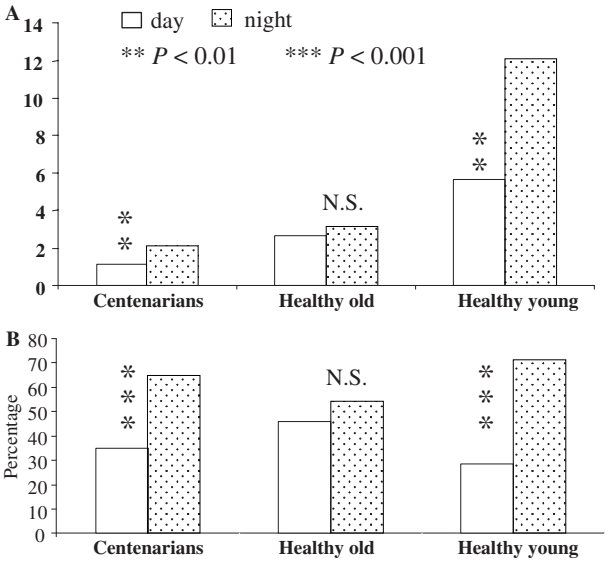

Fig. 2. Urinary excretion of 6-hydroxymelatonin sulfate (aMT6s) in centenarians and healthy old and young controls. (A) Diurnal and nocturnal aMT6s excretion. (B) Diurnal and nocturnal aMT6s excretion expressed as percent of the total amount.

controls, while in healthy old subjects the two values were not significantly different (Fig. 2B).

Discussion

Pineal secretory activity undergoes significant changes with age. Indeed, a progressive lowering of nocturnal melatonin peak becomes apparent when going from adulthood to senescence. Our data confirm this age-related decline, both in terms of nocturnal melatonin peak and of melatonin index, i.e. the total melatonin secretion during the 24 hr cycle. This finding agrees with many publications, although a few authors failed to find any age-related melatonin decline [37, 38], probably because of the different study conditions. The possible mechanisms by which melatonin secretion declines with age may depend on some regressive changes such as the calcification of the pineal gland, although these changes alter neither the histology nor the enzymatic activity of the pineal gland [39, 40]. An increased age-related plasma melatonin clearance has been suggested, but the finding of the reduced urinary excretion of aMT6s with age [41], persisting also in centenarians, as we have found in our study, and the strong correlation between plasma melatonin and urinary aMT6s, make this hypothesis less probable.

Although an age-related decline of the pineal secretory capacity cannot be ruled out [42], it seems likely that a primary cause of the marked decrease in melatonin secretion might be the reduction of the number and/or sensitivity of noradrenergic receptors on the pinealocytes [43]. Furthermore, recent evidence also suggests a dramatically age-related decline in N-acetyltransferase activity, a key step in melatonin synthesis [44].

The existence of significant relationship between the nocturnal melatonin peak and both the subjects' age and the MMSE score, suggests that the pineal secretion can be affected not only by the aging process, but also by the degree of cognitive impairment [45]. Indeed, senile dementia is often associated to alterations of some periodic functions, such as those in neuroendocrine system, body temperature and the rest-activity cycle [46, 47], suggesting the existence of damage to the hypothalamic SCN neurons [48, 49]. However, in spite of the changes of the hypothalamic SCN often described in senile dementia, the circadian fluctuation of plasma melatonin maintained statistical significance during mean cosinor analysis, while in experimental animals the bilateral destruction of SCN results in the suppression of the melatonin circadian rhythmicity [50].

Significantly lower melatonin levels in the cerebrospinal fluid have been found in elderly control subjects and, particularly, in AD patients with Apo E ε 4/4 genotype, a well known risk factor for AD onset and progression [51]. Many experimental and clinical findings suggest that melatonin administration could be beneficial in reducing neuronal loss [31] and for the treatment of sleep disorders and sundowning in AD [47]; however, larger and more controlled clinical trials are needed to establish the real therapeutic role of melatonin in AD.

Melatonin signal is closely dependent on the amplitude of nocturnal melatonin secretion, which is probably genetically determined [52]. As suggested by several groups [53, 54], the decrease of melatonin amplitude and/or of the duration of its nocturnal peak could be responsible for an internal temporal desynchronization with a consequent poor adaptability to the internal and external environmental changes and thus to the deterioration of health. Therefore, it is interesting that in centenarian subjects, a highly selected group considered as a good expression of successful aging, the nocturnal excretion of aMT6s was significantly higher than diurnal levels; when expressed as percent of the total 24 hr amount the diurnal and nocturnal

excretion rate were quite similar in centenarians and young subjects while no differences between the two urine samples were found in 'normal' aging, suggesting a certain maintenance of the circadian organization of melatonin secretion in the former but not in the latter.

In conclusion, melatonin secretion exhibited an age-related reduction of the amplitude of the circadian fluctuations and a selective impairment of the nocturnal peak in elderly people and even more in old demented patients. Thus pineal secretion may be modified by both the age and the degree of cognitive impairment. The total aMT6s excretion rate, a major melatonin metabolite, clearly declined with age. However, the physiological circadian periodicity of melatonin secretion was maintained in centenarians but not in aged controls. As melatonin plays an important role both as endogenous synchronizer and as free radical scavenger, the persistence of the physiological circadian organization of melatonin secretion could be relevant for successful aging.

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