Ontogeny and aging of the distal skin temperature rhythm in humans

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Abstract In circadian terms, human ontogeny is characterized by the emergence of a daily pattern, from a previous ultradian pattern, for most variables during the first 6 months of life. Circadian aging in humans is characterized by a phase advance, accompanied by rhythm fragmentation and flattening. Despite an expanding body of literature focused on distal skin temperature, little information is available about the ontogeny and practically nothing about age-related changes in this rhythm. Thus, the aim was to evaluate the degree of maturation and aging of the circadian pattern of distal skin temperature to identify those

parameters that are modified throughout life and could be used to differentiate subjects according to their age. For this, distal skin temperature was measured in 197 volunteers (55 % women), including babies aged 15 days (30 subjects), 1 month (28 subjects), 3 months (31 subjects), and 6 months (10 subjects); young adults aged 19 years (37 subjects); middle-aged persons aged 46 years (27 subjects); older people aged 72 (34 subjects). Circadian system maturation was associated with an increase in amplitude and a reduction in skin temperature during sleep. During adulthood, women showed a more robust pattern (lower fragmentation, and higher night-time temperature, amplitude, circadian function index, and first harmonic relative power); however, these differences were lost with aging, a period of life that was consistently associated with a phase advance of the rhythm. In summary, distal skin temperature pattern can be used as a robust variable to discern between different ages throughout the life.

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Introduction

The circadian system (CS) consists of a network of structures involved in the generation, maintenance, and synchronization of circadian rhythms (Stratmann and Schibler 2006). As occurs with other systems, the CS matures during ontogeny and exhibits a progressive loss

of functionality associated with aging (McGraw et al. 1999; Turek et al. 1995).

Newborn rhythms show an ultradian pattern (with a period of around 2–4 h), which seems to be the result of the expression of an uncoupled multioscillatory CS, which would be coupling by synaptogenesis to reach the circadian oscillation before the 6 months of age emerging as CS matures (McGraw et al. 1999; Weinert 2005; Zornoza-Moreno et al. 2011). During the adulthood, CS is mature, but gender differences appear during this period related to chronotype (Adan and Natale 2002). Finally, aging is associated with a phase advance, fragmentation, and flattening of marker rhythms (robust, easy-to-register, noninvasive, and comfortable circadian rhythm driven by the circadian pacemaker) (Hofman and Swaab 2006; Van Someren et al. 1999).

The importance of the CS is becoming increasingly recognized in clinical medicine. In newborns and infants, delayed circadian maturation may be caused by an early deficiency in exposure to environmental synchronizers or by impaired neural development (Rivkees 2003). In adults and older people, circadian disruption is associated with increased morbidity and mortality due to aging and certain pathologies, such as metabolic syndrome, cancer, cognitive and affective disorders, sleep impairment, and cardiovascular events (Garaulet et al. 2010; Reiter et al. 2007). Thus, the development of reliable and noninvasive means to assess CS functionality in subjects in their own home environment has become an imperative.

To assess the CS, certain marker rhythms have been proposed. The most used marker rhythms are melatonin and cortisol secretion, core body temperature (CBT), and activity (Van Someren 2000). Besides, distal skin temperature (DST) has recently been proposed as a noninvasive, robust, comfortable, and easy-to-register tool for CS assessment (Blazquez et al. 2012; Bonmati-Carrion et al. 2014; Kräuchi 2002; Kräuchi et al. 2005; Martinez-Nicolas et al. 2013, 2014; Romejin and Van Someren 2011; Zornoza-Moreno et al. 2011). Moreover, DST has been suggested as a major regulator of CBT, since DST is the result of peripheral vasodilatation in response to nocturnal sympathetic inhibition (Blazquez et al. 2012; Buijs et al. 2003; Kräuchi et al. 2006; Martinez-Nicolas et al. 2014).

A growing body of evidence indicates that DST is a noninvasive, reliable, and comfortable tool to evaluate the CS under various conditions, in both clinical and nonclinical settings and for newborns, healthy young

subjects and subjects suffering from obesity and metabolic syndrome, and non-dipping blood pressure pattern (Bandin et al. 2012; Bonmati-Carrion et al. 2014; Kräuchi 2002; Martinez-Nicolas et al. 2013, 2014; Zornoza-Moreno et al. 2011). However, little information is available about its ontogeny and next to nothing about age- or sex-related changes in DST.

The aim of this study was to determine, together with its masking factors, distal skin temperature rhythm maturation and aging in order to identify the most reliable rhythmic parameters of the temperature rhythm to differentiate subjects according to their age.

Material and methods

Participants

The study consists of two parts. The first one is focused on the ontogeny of the circadian rhythm of distal skin temperature using the data from 15-day-old to 6-month-old babies, since DST rhythm has been described to appear in the third month of life (Zornoza-Moreno et al. 2011). Aging will be addressed in the second part of the study, using young, middle-aged, and elder volunteers.

Thus, a total of 197 Caucasian volunteers, ranging from newborns (15 days of age) to older people (85 years of age) of both sexes (55 % female), participated in this cross-sectional study. All the recordings were made from October to April. The population was divided in seven groups according to age: (1) 30 newborns (50 % female, 15 days old), (2) 28 1-month-old babies (53.6 % female), (3) 31 3-month-old babies (54.8 % female), (4) 10 6-month-old babies (60 % female), (5) 37 young adults (51.4 % women, 19 ± 1 year old), (6) 27 middle-aged adults (77.8 % women, 46 ± 2 years old), (7) 34 older people (41.2 % women, 72 ± 1 year old). Young and middle-aged groups were university students and workers, respectively. Women from these groups showed regular menstruation, while older people were retired. All participants were healthy, no shift or night workers, mainly sedentary (less than 2 h of exercise per week), with no physical conditions that disturbed their sleep (e.g. asthma, restless legs, and sleep apnea) and who did not travel recently across multiple time zones according to their answers in a personal interview. During the experiments, which took place in the cities of Murcia and Toledo (Spain), the participants were

encouraged to maintain their usual life styles. The study abides by the bioethical principles set out by the Declaration of Helsinki. Data from the volunteers were included in a database and were protected according to Spanish law 15/1999 of 13 September. All participants received the appropriate information about the study characteristics and signed an informed consent before their inclusion in the study.

Temperature measurement

All adults wore a Thermochron iButton DS1921H (Dallas, Maxim) for skin wrist temperature measurement, which had a precision of ± 0.125 °C. This temperature sensor was placed on the wrist of the nondominant hand over the radial artery and isolated from the environmental temperature by a double-sided cotton sport wrist band, as previously described (Martinez-Nicolas et al. 2013). The babies wore the device inside a sock to isolate it from the environmental temperature, as previously described (Zornoza-Moreno et al. 2011). All devices were programmed to sample once every 10 min, and they were worn for three (in the case of babies) or seven consecutive days (remaining groups).

Data analysis

Circadianware™ v7.1.1 (Sosa et al. 2010) was used to analyze the distal skin temperature rhythm for each subject. This software enables the calculation of the main rhythmic parameters of a time series. Briefly, the Cosinor was applied to calculate the mesor, amplitude, acrophase (Fig. 1a), and percentage of variance explained by the cosine wave (%V). A Rayleigh test, chi-square periodogram, Fourier analysis with the first 12 harmonics, and the circadianity index (calculated as first harmonic power/sum of 12th harmonics power, it measures the relative power of the circadian component compared with the ultradian components) were also calculated, in a similar way to that described before (Zornoza-Moreno et al. 2011). All above described parameters, excepting chi-square periodogram, assume that the rhythm is sinusoidal, and all of them have been previously used for assessing the coupling of the CS.

Because the wrist temperature (WT) rhythm has a shape that is far from sinusoidal in many subjects, a nonparametric analysis was performed as described by Van Someren et al. (1999). This analysis permits the calculation of interdaily stability (IS, the consistency of

Fig. 1 Phases and variables for DST daily pattern. Parametric analysis is detached in part A, where an example from a DST pattern with the adjusted cosine wave and its parameters (mesor, amplitude, and acrophase) is shown. Part B summarized the non-parametric analysis on an example of DST placing on the wave the characteristic timings (TL10, TM5 and TL2) and their mean value (L10, M5 and L2). Both parts are divided in four sections according to local time: morning decrease (i), afternoon secondary peak (ii), evening decrease (iii), and night plateau (iv)

the 24-h rhythmic pattern over days), intradaily variability (IV, the rhythm fragmentation), the hourly average during 2 consecutive hours, along with the minimum temperature (L2) and its timing (TL2), the hourly average during 5 consecutive hours, along with the maximum temperature (M5) and its timing (TM5), the hourly average during 10 consecutive hours, along with the minimum temperature (L10) and its respective timing (TL10), and the relative amplitude (RA) determined as the difference between M5 and L10, divided by the sum of M5 and L10, and the circadian function index or CFI, as previously described (Ortiz-Tudela et al. 2010). Phase markers (TL2, TM5, and TL10) and their respective average temperature values (L2, M5, and L10) are outlined in Fig. 1b. All the variables previously described were individually obtained from each subject; then they were averaged according to age group to obtain its mean value.

In order to facilitate the description of the results and as it can be observed in Fig. 1, the mean pattern of DST rhythm was divided in four main phases: (i) a morning decrease (0800–1500 hours) starting immediately after awakening, (ii) a secondary peak in the afternoon, coinciding with the postprandial period (1500–1800 hours), (iii) an evening decrease (1800–2300 hours), associated with the “wake maintenance zone”, and (iv) a nocturnal plateau (2300–0800 hours) of high DST, associated with the main sleep period.

In order to differentiate age groups, the interrelationship among variables was separated by a principal component analysis. With the most explanatory variables, a bivariate diagram was performed using Qp and TL10. However, the obtained diagram did not explain linearly both processes. Thus, two additional principal component analyses were performed (one for ontogeny and other for aging), and the variables with the strongest relationships with chronological age and the least possible interrelationship between them were selected. Thus, ontogeny relative amplitude and mesor were selected while mesor and acrophase were selected for aging.

Finally, the statistical computing software R 3.0.0 was used to perform a one-way ANOVA to evaluate differences among age groups, followed by a Bonferroni post hoc pairwise comparison, which is appropriate. To analyze the effects of sex and age and any possible interaction between them, a two-way ANOVA was performed. When only two groups were compared (for example, to test for differences by gender in each age group), a Student's *t* test was performed. In all cases, $p < 0.05$ was considered to be statistically significant.

Results

To check the correspondence between parametric and nonparametric indexes, correlation analyses were performed, which found no equivalence for regularity in the rhythmic pattern, since IS was only slightly correlated with the Rayleigh test ($r = 0.27$, $p < 0.001$). Rhythm fragmentation, calculated as IV, was significantly correlated with the circadianity index ($r = -0.40$, $p < 0.001$). Relative amplitude showed an almost perfect correlation with cosinor amplitude ($r = 0.98$, $p < 0.001$), whereas the timing of M5 (TM5) was closely correlated with the cosinor acrophase ($r = 0.65$, $p < 0.001$). Thus, the pairs IV-circadianity index, RA-cosinor amplitude, and TM5-

cosinor acrophase can be considered to be similar indexes for nonparametric and sinusoidal fitting procedures, respectively. However, it should be noted that the Rayleigh test and IS measure different aspects of regularity.

Distal skin temperature exhibited a daily rhythm from the very first days of life (Fig. 2). From 3 months of age onwards, this variable maintained a generally very stable daily pattern throughout the rest of life. During ontogeny, a progressive increase in circadian amplitude was observed (Fig. 2a–d), whereas aging is characterized by a gradual phase advance and the loss of the evening decrease associated with the wake maintenance zone (Fig. 2e–g).

Circadian maturation between 15 days and 6 months of age was associated with a progressive decrease in the mesor of DST, together with a significant reduction in nocturnal M5 and a more pronounced decrease in diurnal L10. The ontogeny of DST was also related with a significant increase in the amplitude of the 24-h circadian component and the circadianity index. A significant increase was also observed in the case of the IS, together with a decrease in rhythm fragmentation (IV). However, no significant changes were observed in any of the circadian phase markers: TL10, TM5, cosinor acrophase, or TL2 (Table 1, Fig. 2a–d).

Contrary to the changes observed in the ontogeny of the DST rhythm, aging was characterized by the advance of several phase markers, together with an increase in rhythm stability, without affecting overall amplitude and fragmentation (Table 2, Fig. 2e–g). Significant phase advances were observed in older people, as indicated by the cosinor acrophase and the nonparametric acrophase calculated as TM5. Another significant change associated with age is the loss of the evening decrease in WT, together with a sharper decrease in WT during the morning. These changes affect TL2, which shifts from evening hours in young people and adults to morning hours in aged people. Consequently, the timing of L10 also shows a phase advance with age. An increase in IS was also observed, reflecting the regularity of the lifestyle of aged people with respect to young and mid-aged adults (Table 2).

The general principal component analysis showed a strengthening of the circadian pattern, as assessed by χ^2 periodogram power (Qp) and circadianity index, with weak (circadianity index, $r = 0.321$, $p < 0.001$) to moderate relationships (Qp, $r = 0.532$, $p < 0.001$), a decrease in the temperature level (evaluated by mesor or M5) with

Fig. 2 Daily patterns of DST. Mean waveforms for 15-day-old newborns (**a**, $n=30$), and at 1 month of age (**b**, $n=28$), 3 months of age (**c**, $n=31$), 6 months of age (**d**, $n=10$), young people (**e**, $n=37$), middle-aged adults (**f**, $n=27$), and older people (**g**, $n=34$). All data are expressed as mean $\pm$ SEM

Table 1 Characterization of ontogeny

	0.5 months	1 month	3 months	6 months
Mesor	34.945±0.089 ^a	34.950±0.090 ^a	33.774±0.142 ^b	33.030±0.251 ^c
Amplitude	0.545±0.083 ^a	0.558±0.089 ^a	0.991±0.108 ^b	1.682±0.234 ^c
Acrophase	04:56±00:40	05:16±00:35	03:28±00:39	03:03±01:10
Rayleigh vector	0.718±0.040	0.763±0.041	0.834±0.037	0.743±0.079
%V	12.704±2.790 ^a	12.231±2.307 ^a	23.070±2.658 ^b	30.268±4.772 ^b
Circadianity index	24.903±4.199 ^a	33.520±4.789 ^{ab}	43.601±3.689 ^b	52.664±6.829 ^b
χ^2 Periodogram	204.210±12.685 ^a	222.630±7.658 ^{ab}	248.903±9.897 ^b	264.100±26.617 ^b
IS	0.468±0.027 ^{ab}	0.443±0.021 ^b	0.562±0.023 ^c	0.597±0.048 ^{ac}
IV	0.218±0.015 ^{ab}	0.290±0.023 ^a	0.237±0.020 ^{ab}	0.146±0.016 ^b
RA	0.017±0.002 ^a	0.017±0.002 ^a	0.029±0.003 ^b	0.049±0.006 ^c
M5	35.664±0.089 ^a	35.620±0.074 ^a	34.942±0.118 ^b	34.898±0.163 ^b
L10	34.466±0.127 ^a	34.468±0.139 ^a	32.973±0.188 ^b	31.685±0.422 ^c
L2	33.587±0.164 ^a	33.626±0.160 ^a	32.035±0.215 ^b	30.700±0.502 ^c
TL10	17:12±00:57	18:00±00:53	16:49±00:54	15:34±01:31
TM5	05:13±00:39	06:38±00:50	05:03±00:40	03:53±00:38
TL2	17:31±00:49	17:34±00:40	15:51±00:40	16:00±00:25
CFI	0.511±0.016 ^a	0.489±0.015 ^a	0.581±0.018 ^b	0.670±0.036 ^b

Mesor, amplitude, acrophase, Rayleigh vector, explained variance by a sinusoidal wave (%V), interdaily stability (IS), intradaily variability (IV), relative amplitude (RA), night, day and wake maintenance values (M5, L10 and L2 respectively) and their corresponding timing (TM5, TL10, and TL2 respectively), circadian function index (CFI), circadianity index, and the power of the chi square periodogram for a period of 24 h (Qp) for the four groups of neonates (15 days, 1 month, 3 months, and 6 months of age). All indexes are expressed as mean±SEM, and different letters indicate significant differences among age groups (one-way ANOVA and Bonferroni post hoc pairwise comparisons, $p<0.05$)

weak negative relationship (mesor, $r=-0.305$, $p<0.001$; M5, $r=-0.395$, $p<0.001$), and a phase advance (according to the acrophase, TL10, TM5, and TL2) with a weak relationship (acrophase, $r=-0.369$, $p<0.001$; TL10, $r=-0.386$, $p<0.001$; TM5, $r=-0.378$, $p<0.001$; TL2, $r=-0.425$, $p<0.001$). Using the two best parameters for circadian pattern strengthening and phase advance (Qp and TL10), a bivariate diagram was plotted in Fig. 3a, showing a circadian pattern enhancement from infancy to middle-aged adults with a tendency to phase advancing with increasing age with the exception of young adults.

To focus on ontogeny of the CS, a principal component analysis was performed for groups from 15 days to 6 months. First, it indicated a decrease in the temperature level (evaluated by either the mesor, L10, M5, or L2) with moderate (M5, $r=-0.501$, $p<0.001$) to strong negative relationships (mesor, $r=-0.730$, $p<0.001$; L10, $r=-0.726$, $p<0.001$; L2, $r=-0.682$, $p<0.001$). The following step found significant increases in both amplitude measurements (amplitude and RA), with a

moderately positive relationship (amplitude, $r=0.557$, $p<0.001$; RA, $r=0.599$, $p<0.001$) as age increased. Using a bidimensional diagram to plot the two best parameters for temperature level and amplitude (mesor against RA), it was possible to discriminate age classes according to a general pattern of temperature rhythm maturation (Fig. 3b). This diagram showed that 15- and 30-day-old infants have quite similar patterns, since Qp, the relative amplitude and acrophase values, overlaps for both groups (Fig. 3a–c). Three-month-old babies evidence more mature patterns, and 6-month-olds are almost mature as can be inferred by the gradual proximity of their values to that observed in adult groups, (Fig. 3a, b) while adult and older groups reduced the values for Qp, RA, and show a phase advance to that observed in previous stages.

To distinguish among the aging groups (young, middle-aged, and older adults), another principal component analysis was performed. On this occasion, it showed that the acrophase, TL2, TM5, and TL10 phase markers were the best correlated with age, evidencing

Table 2 Characterization of aging

	Young	Adults	Older
Mesor	33.220±0.075 ^a	33.689±0.086 ^b	33.643±0.112 ^b
Amplitude	1.086±0.097	0.943±0.105	0.894±0.078
Acrophase	04:51±00:22 ^a	03:12±00:18 ^b	00:37±00:26 ^c
Rayleigh vector	0.690±0.032	0.768±0.030	0.729±0.039
%V	21.512±2.310	18.260±2.709	24.669±2.270
Circadianity index	56.260±3.461	60.320±4.143	54.415±3.248
χ^2 Periodogram	372.184±21.196 ^a	548.333±54.886 ^b	447.029±27.462 ^{ab}
IS	0.357±0.024 ^a	0.297±0.028 ^a	0.452±0.029 ^b
IV	0.184±0.013	0.226±0.020	0.208±0.014
RA	0.032±0.003	0.027±0.003	0.025±0.002
M5	34.573±0.088	34.793±0.107	34.582±0.094
L10	32.399±0.129 ^a	33.065±0.157 ^b	32.981±0.166 ^b
L2	31.774±0.147 ^a	32.449±0.141 ^b	32.101±0.203 ^{ab}
TL10	17:38±00:41 ^a	14:44±00:54 ^b	11:11±00:29 ^c
TM5	04:11±00:16 ^a	04:07±00:19 ^a	01:23±00:28 ^b
TL2	17:14±00:24 ^a	15:11±00:30 ^b	12:36±00:30 ^c
CFI	0.433±0.010 ^{ab}	0.404±0.012 ^a	0.457±0.011 ^b

Mesor, amplitude, acrophase, Rayleigh vector, explained variance by a sinusoidal wave (%V), interdaily stability (IS), intradaily variability (IV), relative amplitude (RA), night, day and wake maintenance values (M5, L10, and L2 respectively) and their corresponding timing (TM5, TL10, and TL2 respectively), circadian function index (CFI), circadianity index, and the power of the chi square periodogram for a period of 24 h (Qp) for the three adult groups (young, middle-aged, and older people). All indexes are expressed as mean±SEM, and different letters indicate significant differences among age groups (one-way ANOVA and Bonferroni post hoc pairwise comparisons, $p<0.05$).

strong (acrophase, $r=-0.628$, $p<0.001$) and moderately negative correlations (TL10, $r=-0.579$, $p<0.001$; TL2, $r=-0.575$, $p<0.001$; TM5, $r=-0.444$, $p<0.001$). In the second step, temperature level (evaluated by mesor and L10) showed significant increases with age, with a weak relationship (mesor, $r=0.354$, $p<0.001$; L10, $r=0.302$, $p<0.001$). Thus, a bidimensional diagram once again plotted the best phase and temperature level markers, acrophase and mesor, to discriminate aged people from the remaining age classes (Fig. 3c), while babies reduced their mesor with almost no changes in acrophase.

A low relative amplitude (lower than 0.2), high mesor (higher than 34.5 °C), low χ^2 periodogram power (lower than 235), and phase delay (TL10 after 1640 hours and acrophase after 0430 hours) correspond with 15-day-old and 1-month-old groups. For 3-month-old group, a little bit higher χ^2 periodogram power (between 235 and 250), a mesor between 33.75 and 33.81 °C, relative amplitude between 0.15 and 0.35, an acrophase placed between 0230 hours and 0430 hours, and TL10 between 1500 hours and

1630 hours were found. In the case of 6-month-old babies, the χ^2 periodogram power was higher in younger babies (between 240 and 290); high relative amplitude (higher than 0.4), low mesor (lower than 33.3 °C), with the TL10 between 1535 hours and 1635 hours, and the acrophase between 0130 hours and 0430 hours were detected. Young adult group showed a χ^2 periodogram power between 350 and 395 units, mesor between 33.15 and 33.3 °C, relative amplitude between 0.25 and 0.40 and delayed phase for TL10 (1650 hours to 1740 hours), and acrophase (0428 hours to 0512 hours). χ^2 periodogram power was high in middle-aged adults (higher than 485), mesor was between 33.6 and 33.8 °C, and relative amplitude was between 0.24 and 0.29 and experienced a phase advance in acrophase (between 0254 hours and 0330 hours) and TL10 (from 1240 hours to 1540 hours) compared to young adults ($p<0.05$). The older adult group reduced its χ^2 periodogram power between 420 and 475; the mesor was between 33.53 and 33.75 °C and relative amplitude between 0.22 and 0.27. Finally, the aged adult group

Fig. 3 Development and aging scatter plot for the wrist temperature pattern. Different ages can be differentiated by χ^2 periodogram power (Qp) and L10 timing (TL10) (a), ontogeny phases (b) can be differentiated by mesor and relative amplitude, while aging (c) can be differentiated by acrophase and mesor. Data are expressed as mean $\pm$ SEM

was phase advanced compared to the other groups with the acrophase earlier than 0105 hours and TL10 earlier than 1305 hours as can be deduced from Fig. 3.

Regarding gender differences in the DST rhythm, M5 was the only variable that showed a statistically significant increase in women, while for the remaining variables, the differences were due to age (Table 3). However, it is interesting to note that when considering both age and gender, statistically significant differences

by gender were only observed in young people (Table 3). In general, all indexes associated with circadian robustness (i.e., cosinor amplitude, %V, IV, RA, M5, and CFI) showed stronger values in young women than in young men, and most circadian parameters (mesor, amplitude, acrophase, IV, RA, L10, L2, TM5, TL10, and TL2) were statistically different between young and aged women. In men, all phase markers (TL2, TM5, and TL10) became phase advanced as age increased, and IS, CFI, and Qp were statistically higher ($p < 0.05$) in older men.

Discussion

We propose that the peripheral skin temperature rhythm can be used as a reliable and simple procedure to discern between age groups in normal-living subjects. Circadian rhythm maturation in babies is characterized by a progressive decrease in DST during the rest period, together with an increase in rhythm amplitude, but with no changes in phase markers. However, strong phase advances without significant changes in rhythm amplitude have been shown to characterize aging. Gender differences were found in adults, with women generally exhibiting better circadian robustness than men; however, these gender differences disappeared in older people.

Our results indicate that cosinor amplitude and RA can be used interchangeably and produce equivalent results. Similarly, cosinor acrophase and TM5 render a good level of agreement when used as phase markers, and the circadianity index and IV can both be considered adequate indexes for rhythm fragmentation. In addition, the weak correlation observed between the Rayleigh test and IS was expected, since the Rayleigh test is a measure of the stability of the acrophase, while IS quantifies the consistency of the pattern between days.

Distal skin temperature is driven by the central pacemaker (Bonmati-Carrion et al. 2014; Kräuchi et al. 2005; Kräuchi et al. 2006; Martinez-Nicolas et al. 2013, 2014; Sarabia et al. 2008) showing a strong endogenous component after mathematical demasking or under constant routine protocol (Kräuchi and Wirz-Justice 1994; Kräuchi et al. 2006; Martinez-Nicolas et al. 2013), although as occurs with core body temperature, melatonin, or sleep rhythms, it is masked by external factors such as activity, sleep, light, environmental temperature, or body position (Cajochen et al.

Table 3 Age-gender differences

	Young men	Older men	Young women	Older women	Global differences
Mesor	33.174±0.030	33.511±0.028	33.266±0.029*	33.833±0.040*	Age ($F=11.71$, $p=0.001$)
Amplitude	0.871±0.023 [#]	0.949±0.026	1.300±0.027* [#]	0.817±0.037*	
Acrophase	04:50±00:08*	24:06±00:07*	04:51±00:07*	01:20±00:10*	Age ($F=54.54$, $p<0.001$)
Rayleigh vector	0.728±0.012	0.719±0.011	0.652±0.011	0.742±0.015	
%V	17.008±0.733 [#]	24.173±0.678	26.016±0.714 [#]	25.378±0.969	
Circadianity index	49.010±1.066 [#]	56.710±0.986	63.510±1.037 [#]	51.138±1.408	
χ^2 Periodogram	333.895±7.784*	430.650±7.197*	410.474±7.576	470.429±1.282	Age ($F=5.23$, $p=0.03$)
IS	0.312±0.057*	0.438±0.082*	0.403±0.076	0.472±0.104	Age ($F=6.97$, $p=0.01$)
IV	0.212±0.004 [#]	0.202±0.004	0.157±0.004* [#]	0.217±0.006*	
RA	0.026±0.007 [#]	0.026±0.007	0.038±0.007* [#]	0.022±0.011*	Age ($F=6.11$, $p=0.02$)
M5	34.242±0.027 [#]	34.505±0.025	34.905±0.026 [#]	34.692±0.035	Sex ($F=13.22$, $p=0.01$)
L10	32.471±0.047	32.759±0.044	32.328±0.046*	33.298±0.062*	Age ($F=9.16$, $p=0.03$)
L2	31.827±0.056	31.840±0.052	31.720±0.055*	32.473±0.074*	
TL10	17:52±00:12*	10:42±00:11*	17:24±00:11*	11:50±00:16*	Age ($F=53.89$, $p<0.001$)
TM5	04:25±00:07*	01:02±00:07*	03:56±00:07*	01:54±00:10*	Age ($F=26.17$, $p<0.001$)
TL2	17:07±00:09*	11:59±00:08*	17:22±00:08*	13:27±00:11*	Age ($F=50.13$, $p<0.001$)
CFI	0.411±0.033* [#]	0.454±0.031*	0.454±0.032 [#]	0.462±0.045	

Mesor, amplitude, acrophase, Rayleigh vector, explained variance by a sinusoidal wave (%V), interdaily stability (IS), intradaily variability (IV), relative amplitude (RA), night, day and wake maintenance values (M5, L10, and L2 respectively) and their corresponding timing (TM5, TL10, and TL2 respectively), circadian function index (CFI), circadianity index, and the power of the chi square periodogram for a period of 24 h (Qp) for young and older people, separated by gender. All indexes are expressed as mean±SEM. * indicates statistically significant age differences for each gender, whereas [#] indicates statistically significant gender differences for each age group as assessed by a Student's *t* test ($p<0.05$). The main effects by age and gender were assessed by a two-way ANOVA (see the last column, labelled as global differences)

2000; Martinez-Nicolas et al. 2013; Reilly and Waterhouse 2009; Wakamura and Tokura 2002). Besides, DST reflects the endogenous rhythm of sympathetic-parasympathetic activation on vessel lumen diameter, in relation to day activity and night rest, respectively (Charkoudian 2003).

Globally, the CS advances its phase and increases the 24-h harmonic power over the years. However, phase advancing was paused by a phase delay in a point between the 6-month-old babies and young adults that presumably could occur during the adolescence (Roenneberg et al. 2007). Regarding the enhancing of the first harmonic, it was consistently increased in all the groups with the exception of older people, probably due to the disruption of the CS by rhythm fragmentation as described for sleep and activity (Van Someren et al. 1999).

Although a 24-h rhythm is already present in newborns at 15 days and 1 month of age, the predominance of ultradian rhythmicities (assessed by the circadianity index) and the very low amplitude suggest that

exogenous masking effects could be primarily responsible for the DST rhythm. Environmental light and temperature, together with the behavioral influences of parental care (Worobey et al. 2009), could induce the weak circadian rhythmicity observed in these newborns. At 6 months of age, most infants show a robust daily pattern, which resembles that of adults. It is characterized by a higher circadianity index and amplitude than that of 15-day-old and 1-month-old babies, reaching values similar to those observed in adults. At 3 months of age, infants show some characteristics of immature circadian rhythms and other characteristics that make them more similar to 6-month-old infants, suggesting that this period can be considered as the inflection point for CS maturation. In fact, 3 months of age is a period characterized by high variability in the rate of circadian maturation. It has been suggested that these differences in circadian rhythmicity development depend on parental care rhythms and environmental conditions (Rivkees 2003; Worobey et al. 2009). Thus, it is likely that infant exposure to bright light during the day, darkness during

the night, and regularity of environmental cues promotes an earlier and more robust appearance of circadian rhythmicity (Rivkees 2003).

Some of the most evident characteristics of DST in youngsters are the significant differences observed in most rhythmic parameters associated with gender, with women exhibiting greater levels of regularity, amplitude, circadianity index and CFI, and lower fragmentation than men. However, these gender differences disappear in older people. Sleep habits and chronotype scores have also been documented to differ between young men and women, with healthier sleep patterns and higher morningness scores in women than in men (Azevedo et al. 2008; Roenneber et al. 2007). Differences in hormonal status could be responsible for such differences, although other factors related to the lifestyle of young people may also be responsible (Roenneber et al. 2007).

Although there is a trend towards reduced amplitude and increased rhythm fragmentation in older people as compared to young adults, these differences were not statistically significant; however, consistent and very significant effects were observed for all circadian phase markers, with progressive phase advances as people age. A decrease in the regularity of lifestyle and thus in IS was also expected; however, our results and those of others fail to support this expectation (Minors et al. 1998). Older people try to adapt lifestyles with increasing regularity that counteracts the CS deterioration that occurs with aging.

As mentioned before, DST failed to show reduced amplitude in older people as expected. It has been published that many circadian rhythms show decreased amplitude with aging under constant routine conditions (Dijk et al. 2000; Harper et al. 2005). This decline may be the result of a decrease in the endogenous component of the rhythm. The fact that our results do not show a statistically significant amplitude reduction can be explained by the compensatory effect of an increase in lifestyle regularity associated with aging. It is true that in our study, no masking factor monitoring was included and thus, the detailed causes of the age-dependent changes cannot be elucidated, including thermoregulatory changes (although thermoregulation itself is also modulated by CS).

Judging from our results and those of others, it is clear that aged individuals show a tendency to

become more morning-oriented than young people (Roenneberg et al. 2007). Again, it can be argued that endogenous (body clock) or exogenous (lifestyle timing) contributions or a combination of both may explain this phase advance. In terms of the endogenous component, there is some evidence of a shorter free-running rhythm for core temperature in older people under a constant routine protocol (Dijk et al. 2000; Harper et al. 2005). However, other studies have failed to find a significant phase advance in aged people when studied under these experimental conditions (Kendall et al. 2001). Although we cannot discard the possibility that their body clock is simply running faster, more reliable explanations take into account the exogenous contribution to circadian rhythms. It is likely that many aged individuals go to bed early because (a) their poor eyesight limits what they can do, (b) they may be exposed to lower light intensities during the afternoon and evening, (c) they become tired more easily during the second half of the day, or (d) their declining mental faculties mean that they get bored more easily (Waterhouse et al. 2012).

Whatever the endogenous or exogenous causes of CS impairment in older people, it is clearly appropriate to include procedures in their lifestyle to increase the dichotomy or contrast between daytime activities during the active phase and to promote sleep during the rest phase. To this end, activities such as exposure to bright light, outdoor physical activity during the day, daytime mental activities, and increased artificial lighting levels (particularly during the afternoon and early evening) should be scheduled. In addition, older people should be advised to sleep in complete darkness (light must be only available as necessary for safety and care needs) and to remain in conditions of darkness or dim light when awakened during the night.

Conclusions

In summary, it has been shown that circadian pattern of DST maturation is associated with an increase in circadian rhythm amplitude and a reduction in skin temperature during sleep, whereas aging is more consistently related to a phase advance. The DST rhythm can be used as a robust variable to discern among maturation and aging groups.

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