Chronobiology of Aging: A Mini-Review

Germaine Cornelissen^a Kuniaki Otsuka^b

^aHalberg Chronobiology Center, University of Minnesota, Minneapolis, Minn., USA; ^bDepartment of Chronomics and Gerontology, Tokyo Women's Medical University, Tokyo, Japan

Key Words

Aging · Caloric restriction · Chronobiology · Circadian rhythms · Clock genes · Metabolic pathways · Suprachiasmatic nuclei

Abstract

Aging is generally associated with weakening of the circadian system. The circadian amplitude is reduced and the circadian acrophase becomes more labile, tending to occur earlier with advancing age. As originally noted by Franz Halberg, similar features are observed in the experimental laboratory after bilateral lesioning of the suprachiasmatic nuclei, suggesting the involvement of clock genes in the aging process as they are in various disease conditions. Recent work has been shedding light on underlying pathways involved in the aging process, with the promise of interventions to extend healthy life spans. Caloric restriction, which is consistently and reproducibly associated with prolonging life in different animal models, is associated with an increased circadian amplitude. These results indicate the critical importance of chronobiology in dealing with problems of aging, from the circadian clock machinery orchestrating metabolism to the development of geroprotectors. The quantitative estimation of circadian rhythm characteristics interpreted in the light of time-specified reference values helps (1) to distinguish effects of natural healthy aging from those associated with disease and predisease; (2) to detect alterations in rhythm characteristics as markers of increased risk before

there is overt disease; and (3) to individually optimize by timing prophylactic and/or therapeutic interventions aimed at restoring a disturbed circadian system and/or enhancing a healthy life span. Mapping changes in amplitude and/or acrophase that may overshadow any change in average value also avoids drawing spurious conclusions resulting from data collected at a fixed clock hour. Timely risk detection combined with treatment optimization by timing (chronotherapy) is the goal of several ongoing comprehensive community-based studies focusing on the well-being of the elderly, so that longevity is not achieved at the cost of a reduced quality of life.

© 2016 S. Karger AG, Basel

Introduction

The search for interventions that can increase the human life span by preventing the development of age-associated diseases and slowing down natural biological

Dedicated to the memory of Franz Halberg, the father of chronobiology, whose vision and pioneering work recognized the importance of rhythms as the indispensable control in all we do. Much of the work presented herein represents his enormous contribution to the field, obtained well before clock genes placed circadian rhythms on an undisputable molecular basis. For additional references to work with Franz Halberg not cited herein, see his completed bibliography posted on our website.

changes as a function of age is not new. At the end of the 18th century, Christoph Wilhelm Hufeland, the private physician and friend of Goethe, Schiller, and Herder, published his famous *Makrobiotik oder Die Kunst, das menschliche Leben zu verlängern* (*Macrobiotics or The Art of Prolonging Human Life*) [1], which earned him extraordinary popularity. Already then, he referred to what we now call the circadian period as ‘the unity of our natural chronology’ [2]. Concerns about dietary composition, however, should not overshadow concerns about the circadian timing and frequency of meals, factors reviewed herein to influence health and longevity. While adding ‘years to life’ is important, it should not be done without consideration for adding ‘life to years’, in other words to make sure ‘to age well’ and to reach a satisfying old age both physically and mentally.

To date, caloric restriction is the only known intervention capable of consistently and reproducibly prolonging life in several animal models [3]. The observation that laboratory rats not only live longer but also have fewer age-associated diseases when their food intake is restricted dates back to the 1930s [4]. Caloric restriction studies largely contributed to the birth of chronobiology, as reviewed elsewhere [5]. By trying to answer the question whether adrenocortical activation could be used to treat breast cancer and prolong life, Halberg documented the importance of the feeding schedule for the circadian rhythm of eosinophil counts. He showed that competing synchronization of lighting and feeding schedules can bring about phase differences leading to controversial results [6]. These experiments revealed that food restriction in mice lowers circulating eosinophil counts and amplifies their circadian rhythm [7] (online suppl. fig. S1; see www.karger.com/doi/10.1159/000450945 for all online suppl. material), thereby illustrating the importance of another rhythm characteristic, the circadian amplitude. Today, a growing body of work at the molecular level suggests reciprocal links between nutrient-sensing pathways and circadian clocks [8].

Modern lifestyle perturbs the human circadian system in 3 primary ways: shift work, exposure to prolonged hours of artificial light, and erratic eating patterns [9]. Circadian disruption is detrimental to health and can lead to metabolic diseases such as diabetes and obesity [8]. Circadian clocks are tightly coupled to cellular metabolism and respond to lighting and feeding schedules. The beneficial impact upon health of strategies such as a regular lifestyle, caloric restriction, and regular exercise likely stems from their effect on strengthening and maintaining synchrony of the circadian system.

This mini-review makes the case for a chronobiologic approach to the study of aging, not just to help unravel underlying molecular mechanisms, but first and foremost to help distinguish effects of natural healthy aging (senescence) from those associated with disease and pre-disease (senility). Senescence or primary aging involves detrimental time-related changes relatively independent of trauma and acquired disease. Differentiating changes associated with senescence from those related to senility is not easy. Many human studies of aging rely on a cross-sectional design, which provides fairly quickly information regarding age trends from different age cohorts, but differences between age groups include confounding factors such as an effect of birth cohort and censoring due to the higher incidence of mortality and disease with increasing age. A longitudinal design overcomes these limitations but requires a much longer time to obtain results. In view of their shorter life span, most longitudinal studies have been carried out in animal models such as mice and rats. A major difficulty in separating human senescence from senility also stems from the lack of well-defined criteria for health status, usually defined negatively as the absence of disease. We show that the derivation of time-specified reference values in clinically healthy people qualified by gender, age, and ethnicity, accounting for circadian (and other) rhythmic variation, is a step toward defining health positively – useful to study both natural aging and to assess disease risk, aiming at primary prevention.

Mapping Trends with Age in Clinical Health

The first indication of a damping of circadian rhythms with advancing age came from studies by Franz Halberg in the 1950s, when he showed that both the rhythm-adjusted mean [midline estimating statistic of rhythm (MESOR)] and the 24-hour amplitude of rectal temperature decreased with aging in the I strain of mice [10]. Later, a decrease in circadian amplitude was also found in the intraperitoneal temperature telemetered longitudinally in Sprague-Dawley rats, and in the body core temperature of the aging stroke-prone Okamoto (SHR-SP) rat, where it was accompanied by an advance of the circadian acrophase (timing of overall high values recurring each day) [11]. Studies on the SHR-SP rat further documented a decrease in the circadian amplitude of the blood pressure rhythm with advancing age.

To investigate the role of neurotransmitters in mechanisms of development, maturation, and aging, changes in

circadian rhythm characteristics of brain monoamines were examined in specific brain regions of male Wistar rats of different ages [12]. A decrease in the circadian amplitude of dopamine, norepinephrine, and 5-hydroxytryptamine was almost invariably observed in the older age cohorts (online suppl. fig. S2). Since at 24 months of age, the animals were not truly senescent, the circadian amplitude can be expected to further decrease in older animals [12].

In clinically healthy men and women, a reduced circadian amplitude of urinary catecholamines was observed as a function of age, together with a decrease in MESOR [13]. In clinically healthy women of 3 age groups, the circadian amplitude of several hormones decreased with age, notably of circulating dehydroepiandrosterone sulfate, cortisol, prolactin, and aldosterone [14] – results that have been replicated in several other studies. The circadian amplitude of urinary and circulating melatonin was also reduced in older subjects. In clinical health, the circadian amplitude of plasma renin activity was reduced in older women as compared to younger women. The circadian amplitude of serum aldosterone was also reduced in 70- to 78-year-old women, but not yet in 60- to 69-year-old women [15]. Acrophase and amplitude charts of circadian and other rhythms of major hormones, vital signs, and other physiological variables serve as useful reference standards in studies of aging, with additional consideration of factors such as altitude and area of residence (rural vs. urban) [16].

In accordance with others, we found that in different populations of clinically healthy subjects, systolic blood pressure reaches a maximum around 80 years of age, whereas diastolic blood pressure starts decreasing around 50 years of age [17], as illustrated for Caucasians in online supplementary figure S3. The dampened circadian variation of blood pressure with advancing age is accompanied by an increased prominence of both ultradian (higher-frequency) and infradian (lower-frequency) components and a circadian acrophase advance of about 2 h [18] (online suppl. fig. S4). The increased prominence of infradian components with advancing age reflects an increased day-to-day variability in the circadian characteristics of blood pressure, in part accounted for by increased about-weekly variation and modulations by long-period cycles present in space weather, such as the about 1.3-year and 11-year cycles characteristic of solar wind speed and solar activity, respectively [19], observed also in a longitudinal record of daily urinary excretion of 17-ketosteroids [20].

Within the scope of our International Chronome Ecology Study Group on Heart Rate Variability (ICE-

HRV) and our project on the Biosphere and the Cosmos (BIOCOS) [16], electrocardiographic records and ambulatory blood pressure and heart rate records have been collected around the clock for 7 days in different geographic locations in patient populations and in clinical health. The time structure was mapped in humans of both genders as a function of age spanning the entire life span. In particular, comprehensive medical assessments carried out in several communities [16] showed that cognitive changes in the elderly may precede rather than follow a decline in sleep quality, that short-term time estimation (10 s) is related to cognitive function in the elderly, and that even mild depression is associated with larger day-to-day blood pressure variability, including a more prominent weekly variation in systolic blood pressure. We learned that in addition to a decreased circadian amplitude and an advanced acrophase of blood pressure, the circadian period is more likely to deviate from 24 h in the elderly. Apart from providing the needed data to derive time-specified reference values qualified by gender and age, these projects led to a better understanding of relationships between cardiovascular function, sleep, mental health and cognition, and quality of life more generally, thereby allowing the timely institution of prophylactic measures, shifting the focus from rehabilitation to prehabilitation medicine (primary prevention).

Caloric Restriction, Longevity, and Circadian Rhythms

Caloric restriction is the only intervention that has been repeatedly demonstrated to prolong life spans across taxa [8] and to reduce cancer incidence in mammals [3]. As compared to BALB/c mice fed *ad libitum*, mice fed during the first 4 h of the light or of the dark span had a larger circadian amplitude of rectal temperature, corticosterone, and liver glycogen. In this and subsequent studies, the amplitude of circadian rhythms was generally increased when food intake was restricted to a single daily ‘meal’ [2, 21]. Meal-feeding altered aspects of carbohydrate and lipid metabolism. It brought about internal desynchronization, as circadian rhythm characteristics of different variables underwent different changes as a function of the timing of the ‘meal’ in relation to the daily lighting regimen. The acrophase of some but not all circadian rhythms was altered depending on the timing of food availability, making it possible to manipulate relations between rhythms in a predictable way.

To determine whether effects of ‘meal’ timing on circadian rhythms were relevant to the well-known beneficial effect of food restriction on life span, additional studies were designed to separate the possible role of rhythm alteration from that of food restriction itself. Female CD2F1 mice allowed to feed *ad libitum* throughout their life were compared to 3 other groups undergoing lifelong restriction to about 75% of *ad libitum* intake, either in the early light or dark span, or receiving 6 smaller ‘meals’ at about 2-hour intervals during darkness (to approximate the pattern of *ad libitum* feeding) [22]. The overall mean life span or the 10th-decile life span did not differ between the 3 restricted groups [22]. The life span was shorter and tumors (mostly mammary) appeared sooner and were more prevalent in the group fed *ad libitum* than in the restricted groups, with no differences between the 3 restricted groups. Restricted feeding was associated with a larger circadian amplitude of telemetered body core temperature, more so with single than with multiple ‘meals’. Suggested factors contributing to the effect of food restriction on survival consisted of a lower body temperature, a reduced overall metabolic rate, and an increased circadian amplitude [22].

In humans, short-term studies of single daily meals, consumed as fixed 2,000 kcal or by free choice, showed a relative weight loss on ‘breakfast only’ but not on ‘dinner only’ [5]. A greater weight loss at ‘breakfast only’ versus ‘dinner only’ is in accordance with circadian variation in diet-induced thermogenesis, which is higher in the morning than in the evening in healthy people [23]. Weight loss on ‘breakfast only’ was smaller on a free-choice meal, even though subjects consumed fewer than 2,000 kcal on a free-choice meal. This apparent paradox may reflect the existence of distinct networks of hypothalamic neurons governing consummatory and appetite behaviors [24, 25]. Other studies showed that caloric restriction and physical exercise improved biomarkers of human aging such as body mass index, blood pressure, glucose, insulin, and lipids, and decreased the risk of aging-associated diseases such as diabetes and cardiovascular conditions [26].

As reviewed elsewhere [5], body temperature, a biomarker of longevity, is decreased by prolonged caloric restriction in humans. Lower caloric intake has been associated with a longer life span in Okinawans, as well as with reduced body weight, blood pressure, blood cholesterol, and blood glucose – variables related to major killer diseases. It has been suggested that caloric restriction and low body temperature may increase life spans synergistically and independently [5]. A larger body mass index

correlated negatively with the circadian amplitude of blood pressure in some studies. A lowered metabolism being implicated in obesity, meal timing – if not overall caloric restriction – presents itself as an appealing lifestyle option available to increase metabolism and reduce body weight.

While caloric restriction and physical exercise are the only known nonpharmacologic interventions capable of delaying aging in rodents and primates, efforts have recently been made to evaluate the relative merits of pharmacologic interventions using geroprotectors as a key stage toward clinical applications [26]. Despite limited clinical investigations performed thus far, the mTOR inhibitor RAD001 (everolimus) was reported to have beneficial effects on immunosenescence in elderly volunteers [26]. The oral antidiabetic drug metformin, which activates AMPK in the liver, was also reported to decrease cardiovascular disease risk, cancer incidence, and overall mortality as compared to other antidiabetic drugs [26, 27]. Other candidate geroprotectors include glycolysis inhibitors, inhibitors of the GH/IGF-1 axis, activators of the sirtuin pathway, inhibitors of inflammatory pathways, and modulators of epigenetic pathways [27]. By identifying essential pathways, it is believed that aging interventions will delay and prevent disease onset for many chronic conditions of adult and old age [27]. As geroprotectors are being developed for human use, one should bear in mind that several overlapping pathways likely contribute to the aging process, and that circadian rhythms are likely to play a role at all stages of geroprotectors’ development. This includes the determination of optimal administration times, reaching a balance between higher efficacy and lesser toxicity. Testing should preferentially be done on actual outcomes rather than being limited to physiologic biomarkers.

Suprachiasmatic Nuclei, Circadian Rhythms, and Aging

In the mid-1970s, experiments were performed in Halberg’s laboratory involving lesioning of the suprachiasmatic nuclei of male inbred Fischer rats, followed several weeks later by implantation of a sensor for continuous monitoring of their intraperitoneal temperature. The suprachiasmatic nuclei are a small brain area of roughly 20,000 neurons situated in the hypothalamus, directly above the optic chiasm. When both suprachiasmatic nuclei were destroyed, the circadian rhythm in telemetered

temperature from freely moving animals exhibited a great amplitude reduction and a circadian acrophase advance, the same features as those observed during the aging process. An increase in amplitude with no change in acrophase of the circadian temperature rhythm was associated with a unilateral lesion of the suprachiasmatic nuclei [28] (online suppl. fig. S5), a result later independently replicated in hamsters.

Lesioning of the suprachiasmatic nuclei was later performed in adult brown house mice of both sexes to determine any effect on the prominent circadian rhythm of the mitotic index of the corneal epithelium. A large reduction in circadian amplitude was again demonstrated. These results led Halberg to conclude that ‘this hypothalamic area integrates the composite of information from within and without the body, i.e., matching internal schedules to changes in external ones and vice versa’. Persistence of the circadian rhythmicity of [³H]-thymidine incorporation into the DNA of different organs (tongue, esophagus, gastric stomach, and colon) and of the mitotic index of the corneal epithelium of female BD2F1 mice after bilateral lesioning of the suprachiasmatic nuclei was later demonstrated [29]. The most consistent result was a phase advance in the rhythms in cell proliferation in the tongue, esophagus, gastric stomach, colon, and corneal epithelium, and a reduction in the circadian amplitude detected in the tongue, esophagus, and corneal epithelium.

The increase in amplitude found for the stomach, colon, and serum corticosterone suggests that the suprachiasmatic nuclei represent but one cog in the overall ‘clock’ mechanism, however important it may be. It has been pointed out that the transcriptional/posttranscriptional delayed feedback loop cannot account for all circadian rhythms in cells [30]. It was suggested that a complementary nontranscriptional-transcriptional coordination mechanism may interact with the classical transcriptional-transcriptional one, and that NAD, involved in energy metabolism, may be involved in their interaction [31]. The presence of intact food anticipatory activity in suprachiasmatic nucleus-ablated rodents or those lacking functional circadian oscillator genes [9] also points to yet unidentified genes and circuits in eating pattern determination. Based on multiple lesion studies performed on animal models with ablation at the level of hypothalamic, corticolimbic, and brainstem structures and adrenals, it has been suggested that circadian coordination is achieved by means of a distributed, decentralized system of oscillators, with contribution in gain setting by the metabolic hormones ghrelin and

leptin [32]. The suprachiasmatic nuclei may also be involved in the coordination of other-than-circadian rhythms, as suggested by a circaseptan amplification in dentin accretion after ablation of the suprachiasmatic nuclei in Wistar rats [33].

The relative independence of the gut from the suprachiasmatic nuclei deserves further investigation. Stimulation of ghrelin production was shown to prolong the life span in 3 different mouse lines, 2 of them with shorter life spans, and to promote better heart health, memory consolidation, and movement [34]. Ghrelin, a hormone involved in hunger, is secreted from the stomach in response to caloric restriction, and coordinates metabolism. Ghrelin signaling activates SIRT1, a NAD-dependent deacetylase, which was reported to interact directly with CLOCK and to deacetylate BMAL1 and PER in mammals [8]. The nutrient sensor AMPK – directly phosphorylating and destabilizing CRY1 and acting upstream of PER – may also affect SIRT1 activity [8]. SIRT1, which declines with age in the suprachiasmatic nuclei [35], is required for high-magnitude circadian transcription of several core clock genes [32]. NAD⁺, generated through de novo biosynthesis from tryptophan, is central for both metabolism and circadian rhythmicity [32]. Through the SIRT1 pathway, treatment for ghrelin resistance may exert a protective effect against brain and other organ/tissue pathologies during the process of aging [34].

Microbiota in the gut may also play a role in host health, and their structure is apparently shaped by diet. Lifelong caloric restriction on both high-fat and low-fat diet was shown to significantly change the overall structure of the gut microbiota of C57BL/6J mice [36]. Caloric restriction enriched phylotypes positively correlated with life span and reduced phylotypes negatively correlated with life span. These calorie restriction-induced changes in the gut microbiota were associated with significantly reduced serum concentrations of lipopolysaccharide-binding protein. The structurally balanced architecture of gut microbiota thus established may hence exert a health benefit to the host via reduction of the antigen load from the gut [36].

Circadian Rhythms, Metabolism, and Aging Signaling Pathways

Most physiological variables are eminently circadian periodic, including those involved in metabolism that are important to ensure that development, survival, and

Fig. 1. Aging is generally associated with a weakening of the circadian system. The circadian amplitude is reduced and the circadian acrophase becomes more labile, tending to occur earlier with advancing age. Similar features have been observed in the experimental laboratory after bilateral lesioning of the suprachiasmatic nuclei. These results suggest the involvement of clock genes in the aging process, as they are in various disease conditions, which are associated with circadian rhythm disruption. Caloric restriction has been consistently and reproducibly associated with healthy life

prolongation in several animal models. A regular routine and physical exercise also help maintain a strong circadian system. To these natural lifestyle options, relative merits of pharmacologic interventions using geroprotectors are being investigated toward clinical applications, but side effects need to be kept in mind. In the presence of overt disease, treatment can be optimized by timing to restore a healthy time structure as a means toward health recovery. ©Halberg Chronobiology Center. SCN = Suprachiasmatic nucleus.

reproduction remain synchronized to environmental changes along the 24-hour scale. Disruption of the circadian system has been linked to increased disease risk, leading to metabolic diseases such as diabetes and obesity. Even in the absence of disease, aging is accompanied by a decline in circadian rhythmicity. Sleep fragmentation, which contributes to a weakened circadian system, was associated with a higher mortality risk in the Rotterdam Study [37]. An age-related decline in circadian organization may account for the reduced amplitude and the in-

creased scatter of acrophases observed in many physiological variables in the elderly.

Circadian clocks are tightly coupled to cellular metabolism [8] and respond to lighting and feeding cycles, as originally shown by Franz Halberg [6]. Meal timing affects both circadian rhythms and metabolism [6, 23]. Caloric restriction achieved by means of intermittent energy restriction or time-restricted feeding reportedly forestalled and even reversed disease processes such as various cancers, cardiovascular conditions, diabetes, and

neurodegenerative disorders [9]. Proposed mechanisms involved include adaptive stress responses, bioenergetics, inflammation, and improved repair and removal of damaged molecules and organelles [9] (fig. 1).

mTORC1, involved in the latter mechanism, is a protein complex that functions as a nutrient/energy/redox sensor. Interest in its upstream signaling arose from its relation to aging: inhibition of mTORC1 was associated with a marked prolongation of life spans in several model species. Carbohydrate consumption was found to activate mTORC1 by means of the insulin growth factor pathway (glucose also activates mTORC1 via Rag GTPases, independently of insulin signaling). Inhibition of mTORC1 was reported to help with autophagy, to increase respiration in the mitochondria to protect against reactive oxygen species, and to conserve stem cells in their undifferentiated condition. Circadian rhythms in phosphorylation of known mTOR targets in the liver, heart, and spleen from wild-type mice are reportedly disrupted in the tissues of BMAL1 knockout mice [38]. BMAL1 deficiency is associated with premature aging and reduced life span, as is increased mTOR signaling. mTORC1 activity is increased upon BMAL1 deficiency both in vivo and in cell culture [38]. Treatment with the mTORC1 inhibitor rapamycin increased the life span of *Bmal1*^{-/-} mice by 50% [38]. In mice, prenatal deletion of *Bmal1*, a core circadian clock gene, disrupts clock-dependent oscillatory gene expression and behavioral rhythmicity coincident with reduced body weight, impaired hair growth, abnormal bone calcification, eye pathologies, neurodegeneration, and a shortened life span [39]. Yet, mice in which the gene is knocked out after birth do not exhibit many of these aging-related phenotypes, suggesting that the circadian clock gene plays different roles during embryogenesis and after birth [39]. These results support the Barker hypothesis that factors impinging on fetal development can influence the expression of aging and life span in adult life [39].

Many of the aging signaling pathways are linked to mechanisms underlying different disease conditions. Blocking disease development constitutes one way to promote healthy aging. The median life span in both male and female wild-type mice of 2 distinct genetic backgrounds was extended by using a transgene to induce apoptosis in p16^{Ink4a}-expressing cells starting at 1 year of age (middle age) as compared to vehicle-treated mice [40]. Cellular senescence is often characterized by expression of p16^{Ink4a}. Senescent cells which accumulate in various tissues and organs over time are thought to play a role in aging. The clearance of p16^{Ink4a}-positive cells was

found to delay tumorigenesis and attenuated age-related deterioration of several organs without apparent side effects. Treated mice had healthier hearts and kidneys, developed cancers later in life, and lived 20–30% longer than untreated mice [40].

Turning back to human studies, it has been suggested that basic clock properties of peripheral cells do not change during aging [41]. Clock properties of fibroblasts cultivated from dermal biopsies of young and older subjects did not differ in period, amplitude, or phase between the 2 groups. A circulating factor may, however, play a role, since measurement of the same cells in the presence of human serum from older donors reportedly shortened period length and advanced the phase of cellular circadian rhythms as compared to treatment with serum from young subjects, suggesting that the phase advance observed in the elderly may be related to sleep fragmentation, changes in eye physiology, or hormonal changes with age that involve a factor possibly acting upon non-suprachiasmatic nuclear regions of the brain and periphery [41].

Following-up on these results, Chen et al. [42] studied the effect of aging on circadian patterns of gene expression in the human prefrontal cortex. This brain area is particularly important for cognitive performance and executive function, and it has been implicated in time estimation, an endogenous timekeeping system different from the circadian system. The authors used a time-of-death analysis to identify transcripts throughout the genome that have a significant circadian rhythm in expression in the human prefrontal cortex. Comparing post-mortem brain samples from clinically healthy subjects in 2 age groups (older than 60 vs. younger than 40 years), they found consistent effects of age on the circadian pattern of expression for PER1 and PER2, specifically involving a phase advance and a reduction in amplitude. They also identified over 1,000 genes that exhibited age-dependent circadian rhythmicity or alterations in rhythmic patterns with aging; some of these genes are known to play a role in rhythm coordination in the suprachiasmatic nuclei.

The identification of molecular pathways involved in aging is an active field of research. A collection of community-curated pathways and knowledge related to aging is available at http://www.agingchart.org/wiki/Main_Page. It contains over 100 pathways, networks, and concept maps on all topics related to aging, from gene-centered pathways to those describing aging processes, age-related diseases, longevity factors, and antiaging strategies. The interactive diagrams can be further explored and up-

Fig. 2. The quantitative estimation of variability taking place within the physiological range leads to refined reference values that help (1) to distinguish effects of natural healthy aging from those associated with disease and pre-disease, (2) to detect alterations in rhythm characteristics as markers of increased risk before there is overt disease, and (3) to individually optimize by timing prophylactic and/or therapeutic interventions aimed at restoring a dis-

turbed circadian system and/or enhancing a healthy life span. Timely risk detection combined with treatment optimization by timing (chronotherapy) is the goal of several ongoing comprehensive community-based studies focusing on the well-being of the elderly, so that longevity is not achieved at the cost of a reduced quality of life. ©Halberg Chronobiology Center.

dated with new contributions. Circadian maps of key elements in this aging chart would be a valuable addition. A database of circadian expression profiles in mammals, based on RNA-seq and DNA arrays quantifying the transcriptomes of 12 mouse organs over time (<http://circadb.hogeneschlab.org/>), already provides useful information.

Rhythms as the Indispensable Control

A decrease in circadian amplitude and an advance in circadian acrophase are general features of senescence, also observed after bilateral lesioning of the suprachiasmatic nuclei. By contrast, caloric restriction, associated with a longer life span, is characterized by an increased circadian amplitude. It thus seems mandatory to assess circadian variation as the indispensable control. Since

differences in amplitude and/or acrophase are not necessarily accompanied by a difference in overall mean value (MESOR), sampling at a fixed clock hour can lead to spurious results [43], as illustrated in online supplementary figure S6 for the case of 1-min time estimation by a clinically healthy man [44]. Circadian rhythmic change can be so large that in several self-measured variables it was more important than that associated with aging over an entire decade [45].

Halberg's extended cosinor method lends itself well to the estimation of circadian (and other) rhythm characteristics, as it does not require data to be equidistant. In addition to rhythm detection, each parameter is estimated with a measure of uncertainty, making it possible to test for differences in amplitude and/or acrophase as well as for differences in MESOR, not only for group comparison but also on an individual basis. The availability of monitoring devices has greatly helped in assembling clinical

data from presumably healthy people, some monitored around the clock for decades by a few dedicated individuals [19]. It thus becomes possible to derive time-specified reference values and reference values for the MESOR, circadian amplitude, and circadian acrophase, further qualified by gender and age. Predictable variation within the physiological range can thus be mapped as a step toward defining clinical health positively and quantitatively (fig. 2).

Establishing chronobiologic standards allows detecting early changes taking place within the physiological range. Deviation from time-specified norms, in turn, can be viewed as an indication for increased disease risk, detected well before there is overt disease. An opportunity then exists to intervene in a timely manner, as ‘prehabilitation’, before there is a need for rehabilitation. Alterations to circadian (and other) rhythm characteristics in the presence of increased disease risk address questions of secondary aging (senility). When acted upon in a timely manner, healthy rhythmic patterns can be restored, and hence existing risks can be reduced, as shown in several clinical trials [16] (online suppl. fig. S7).

Nonpharmacologic or pharmacologic treatment when needed can be optimized by timing (chronotherapy), preferably guided by marker rhythmometry. Using tumor temperature as a marker rhythm, chronoradiotherapy for patients with cancers of the oral cavity led to a faster tumor regression rate and doubling of 2-year disease-free survival rates when it was applied at the time of peak tumor temperature, as compared to 4 or 8 h before or after that time or without consideration of circadian timing (treatment as usual) [46]. Circadian genes have now been linked to tumor suppression and disruptions in circadian genes related to cancer growth [47], the amplitude of circadian rhythms being associated with the prognosis of cancer patients.

In the field of blood pressure, clinical outcome studies in the USA, Europe, and Asia showed that an elevated blood pressure (MESOR-hypertension) is not the only cardiovascular disease risk factor. An excessive circadian amplitude of blood pressure and an acrophase of blood pressure, but not of heart rate, occurring outside the 90% prediction limits of clinically healthy peers matched by gender and age are circadian alterations associated with a greatly increased cardiovascular disease risk (online suppl. fig. S8). In an outcome study, uncomplicated MESOR-hypertension was associated with about 9% morbidity at the 6-year follow-up [19]. Deviation from the norm by 1 or 2 additional features of blood pressure or heart rate variability raised 6-year morbidity

from 9 to 29 and 53%, respectively [19]. Recognizing that different patients present with differently altered circadian profiles of blood pressure (chronodiagnosis), chronotherapy is best adjusted to the chronodiagnosis (chronotheranostics) [43] via truly personalized medicine, since the optimal treatment time differs from one patient to another, as demonstrated in a study by Watanabe et al. [48].

Concluding Remarks

Chronobiologic facts, concepts, and methods are relevant to gerontology and geriatrics. Reference values accounting for rhythms and for changes with gender and age offer new sensitive endpoints as gauges of health and indicators for disease and predisease, making it possible to distinguish the presence of an increased disease risk from healthy aging (online suppl. fig. S9). Effects of interventions such as caloric restriction or any other new geroprotector are readily assessed on an individual or population basis by a chronobiologic interpretation of longitudinal data from physiological monitors. Tracking physiological changes as a function of time helps health maintenance and even health improvement, thus ‘adding life to years’ and not just ‘years to life’.

Acknowledgment

This work was supported by the Halberg Chronobiology Fund, the University of Minnesota Supercomputing Institute, and the A&D Company (Tokyo, Japan).

References

- 1 Hufeland CW: *Makrobiotik, the Art of Prolonging Life*, 2nd English translation. London, printed for J Bell, 1797, p 201.
- 2 Halberg F, Nelson W: Chronobiologic optimization of aging; in Samis H, Capobianco S (eds): *Advances in Experimental Medicine and Biology*. New York, Plenum Press, 1978, vol 108, pp 5–56.
- 3 Weindrich R: The retardation of aging by caloric restriction: studies in rodents and primates. *Toxicol Pathol* 1996;24:742–745.
- 4 McCay CM, Crowell MF, Maynard LA: The effect of retarded growth upon the length of life span and upon the ultimate body size. *J Nutr* 1935;10:63–79.
- 5 Cornelissen G: When you eat matters: 60 years of Franz Halberg’s nutrition chronomics. *Open Nutraceuticals J* 2012;5(suppl 1-M1):16–44.

- 6 Halberg F, Visscher MB, Bittner JJ: Eosinophil rhythm in mice: range of occurrence; effects of illumination, feeding, and adrenalectomy. *Am J Physiol* 1953;174:109–122.
- 7 Halberg F, Visscher MB: A difference between the effects of dietary calorie restriction on the estrous cycle and on the 24-hour adrenal cortical cycle in rodents. *Endocrinology* 1952;51:329–335.
- 8 Tevy MF, Giebultowicz J, Pincus Z, Mazzocchi G, Vinciguerra M: Aging signaling pathways and circadian clock-dependent metabolic derangements. *Trends Endocrinol Metab* 2013;24:229–237.
- 9 Mattson MP, Allison DB, Fontana L, Harvie M, Longo VD, Malaisse WJ, Mosley M, Notterpek L, Ravussin E, Scheer FA, Seyfried TN, Varady KA, Panda S: Meal frequency and timing in health and disease. *Proc Natl Acad Sci USA* 2014;111:16647–16653.
- 10 Halberg F, Bittner JJ, Gully RJ, Albrecht PG, Brackney EL: 24-Hour periodicity and audiogenic convulsions in I mice of various ages. *Proc Soc Exp Biol Med* 1955;88:169–173.
- 11 Halberg J, Halberg E, Regal P, Halberg F: Changes with age characterize circadian rhythms in telemetered core temperature of stroke-prone rats. *J Gerontol* 1981;36:28–30.
- 12 Radha E, Shankaraiah K, Halberg F, Bhaskaran D: Developmental, circadian and aging aspects of dopamine, norepinephrine and 5-HT in rat brain regions; in Redfern PH, Campbell I, Xavier JA, Martin KF (eds): *Circadian Rhythms in the Central Nervous System*. Proc IX Conf IUPHAR, Sat Symp, Bath, England, August 4–5, 1984. London, Macmillan, 1985, pp 199–209.
- 13 Descovich GC, Montalbetti N, Kühl JFW, Rimondi S, Halberg F, Ceredi C: Age and catecholamine rhythms. *Chronobiologia* 1974;1:163–171.
- 14 Nelson W, Bingham C, Haus E, Lakatua DJ, Kawasaki T, Halberg F: Rhythm-adjusted age effects in a concomitant study of twelve hormones in blood plasma of women. *J Gerontol* 1980;35:512–519.
- 15 Cugini P, Scavo D, Halberg F, Schramm A, Pusch H-J, Franke H: Methodologically critical interactions of circadian rhythms, sex and aging characterize serum aldosterone of the female adrenalectomized. *J Gerontol* 1982;37:403–411.
- 16 Otsuka K, Cornelissen G, Halberg F: *Chronomics and Continuous Ambulatory Blood Pressure Monitoring – Vascular Chronomics: From 7-Day/24-Hour to Lifelong Monitoring*. Tokyo, Springer Japan, 2016.
- 17 Cornelissen G, Otsuka K, Halberg F: Blood pressure and heart rate chronome mapping: a complement to the human genome initiative; in Otsuka K, Cornelissen G, Halberg F (eds): *Chronocardiography and Chronomedicine: Humans in Time and Cosmos*. Tokyo, Life Science Publishing, 1993, pp 16–48.
- 18 Gubin D, Cornelissen G, Halberg F, Gubin G, Uezono K, Kawasaki T: The human blood pressure chronome: a biological gauge of aging. *In Vivo* 1997;11:485–494.
- 19 Halberg F, Powell D, Otsuka K, Watanabe Y, Beaty LA, Rosch P, Czaplicki J, Hillman D, Schwartzkopff O, Cornelissen G: Diagnosing vascular variability anomalies, not only ME-SOR-hypertension. *Am J Physiol Heart Circ Physiol* 2013;305:H279–H294.
- 20 Halberg F, Cornelissen G, Bakken EE, Sothorn RB, Schwartzkopff O, Hamburger C: Transyears: new endpoints for gerontology and geriatrics or confusing sources of variability? *J Gerontol A Biol Sci Med Sci* 2004;59:1344–1347.
- 21 Halberg F, Nelson W, Lakatua D, Cadotte L, Haus E: Circadian amplitude increase associated with caloric restriction of mice by single daily meal reduced by ‘pattern-feeding’; in Haus E, Halberg F (eds): *Chronobiology 1982–1983*. Basel, Karger, 1984, pp 490–492.
- 22 Nelson W, Halberg F: Meal-timing, circadian rhythms and lifespan of mice. *J Nutr* 1986;116:2244–2253.
- 23 Bo S, Fadda M, Castiglione A, Ciccone G, De Francesco A, Fedele D, Guggino A, Parasiliti Caprino M, Ferrara S, Vezio Boggio M, Mengozzi G, Ghigo E, Maccario M, Broglio F: Is the timing of caloric intake associated with variation in diet-induced thermogenesis and in the metabolic pattern? A randomized cross-over study. *Int J Obes (Lond)* 2015;39:1689–1695.
- 24 Jennings JH, Ung RL, Resendez SL, Stamatakis AM, Taylor JG, Huang J, Veleta K, Kantak PA, Aita M, Schilling-Scriver K, Ramakrishnan C, Deisseroth K, Otte S, Stuber GD: Visualizing hypothalamic network dynamics for appetitive and consummatory behaviors. *Cell* 2015;160:516–527.
- 25 Nieh EH, Matthews GA, Allsop GA, Presbrey KN, Leppla CA, Wichmann R, Neve R, Wildes CP, Tye KM: Decoding neural circuits that control compulsive sucrose seeking. *Cell* 2015;160:528–541.
- 26 Moskalev A, Chernyagina E, Tsvetkov V, Fedintsev A, Shaposhnikov M, Krut’ko V, Zhavoronkov A, Kennedy BK: Developing criteria for evaluation of geroprotectors as a key stage toward translation to the clinic. *Aging Cell* 2016;15:407–415.
- 27 Longo VD, Antebi A, Bartke A, Barzilay N, Brown-Borg HM, Caruso C, Curiel TJ, de Cabo R, Franceschi C, Gems D, Ingram DK, Johnson TE, Kennedy BK, Kenyon C, Klein S, Kopchick JJ, Lepperdinger G, Madeo F, Mirisola MG, Mitchell JR, Passarino G, Rudolph KL, Sedivy JM, Shadel GS, Sinclair DA, Spindler SR, Suh Y, Vijg J, Vinciguerra M, Fontana L: Interventions to slow aging in humans: are we ready? *Aging Cell* 2015;14:497–510.
- 28 Powell EW, Halberg F, Pasley JN, Lubanovic W, Ernsberger P, Scheving LE: Suprachiasmatic nucleus and circadian core temperature rhythm in the rat. *J Therm Biol* 1980;5:189–196.
- 29 Scheving LE, Tsai TS, Powell EW, Pasley JN, Halberg F, Dunn J: Bilateral lesions of suprachiasmatic nuclei affect circadian rhythms in [³H]-thymidine incorporation into deoxyribonucleic acid in mouse intestinal tract, mitotic index of corneal epithelium, and serum corticosterone. *Anat Rec (Hoboken)* 1983;205:239–249.
- 30 Tomita J, Nakajima M, Kondo T, Iwasaki H: No transcription-translation feedback in circadian rhythm of KaiC phosphorylation. *Science* 2005;307:251–254.
- 31 Yang Y, Jiang Z, Cheng S, Wang Y, Liu Y, Xiao J, Guo H, Li S, Hou W, Wang Z: Nicotinamide adenine dinucleotide: a possible circadian zeitgeber functioning in nontranscription oscillation. *Biol Rhythm Res* 2013;45:429–440.
- 32 Mazzocchi G, Paziienza V, Vinciguerra M: Clock genes and clock-controlled genes in the regulation of metabolic rhythms. *Chronobiol Int* 2012;29:227–251.
- 33 Shinoda H, Ohtsuka-Isaya M, Cornelissen G, Halberg F: Putative circaseptans or other infradian in murine dentin accretion and the suprachiasmatic nuclei. *Neuroendocrinol Lett* 2003;24(suppl 1):208–211.
- 34 Fujitsuka N, Asakawa A, Morinaga A, Amitani MS, Amitani H, Katsuura G, Sawada Y, Sudo Y, Uezono Y, Mochiki E, Sakata I, Sakai T, Hanazaki K, Yada T, Yakabi K, Sakuma E, Ueki T, Nijima A, Nakagawa K, Okubo N, Takeda H, Asaka M, Inui A: Increased ghrelin signaling prolongs survival in mouse models of human aging through activation of sirtuin1. *Mol Psychiatry* 2016, Epub ahead of print.
- 35 Belden WJ, Dunlap JC: Aging well with a little wine and a good clock. *Cell* 2013;153:1421–1422.
- 36 Zhang C, Li S, Yang L, Huang P, Li W, Wang S, Zhao G, Zhang M, Pang X, Yan Z, Liu Y, Zhao L: Structural modulation of gut microbiota in life-long calorie-restricted mice. *Nat Commun* 2013;4:2163.
- 37 Zuurbier LA, Luik AI, Hofman A, Franco OH, Van Someren EJ, Tiemeier H: Fragmentation and stability of circadian activity rhythms predict mortality: the Rotterdam Study. *Am J Epidemiol* 2015;181:54–63.
- 38 Khapre RV, Kondratova AA, Patel S, Dubrovsky Y, Wrobel M, Antoch MP, Kondratov RV: BMAL1-dependent regulation of the mTOR signaling pathway delays aging. *Aging (Albany NY)* 2014;6:48–57.
- 39 Yang G, Chen L, Grant GR, Paschos G, Song WL, Musiek ES, Lee V, McLoughlin SC, Grosser T, Cotsarelis G, FitzGerald GA: Timing of expression of the core clock gene *Bmal1* influences its effects on aging and survival. *Sci Transl Med* 2016;8:324ra16.
- 40 Baker DJ, Childs BG, Durik M, Wijers ME, Sieben CJ, Zhong J, Saltness RA, Jeganathan KB, Casacang Verzosa G, Pezeshki A, Khazaie K, Miller JD, van Deursen JM: Naturally occurring p16^{Ink4a}-positive cells shorten healthy lifespan. *Nature* 2016;530:184–189.

- 41 Pagani L, Schmitt K, Meier F, Izakovic J, Roemer K, Viola A, Cajochen C, Wirz-Justice A, Brown SA, Eckert A: Serum factors in older individuals change cellular clock properties. *Proc Natl Acad Sci USA* 2011;108:7218–7223.
- 42 Chen CY, Logan RW, Ma T, Lewis DA, Tseng GC, Sibille E, McClung CA: Effects of aging on circadian patterns of gene expression in the human prefrontal cortex. *Proc Natl Acad Sci USA* 2016;113:206–211.
- 43 Cornelissen G, Halberg F: Treatment with open eyes: markers-guided chronotherapeutics; in Youan BC (ed): *Chronopharmaceutics: Science and Technology for Biological Rhythm-Guided Therapy and Prevention of Diseases*. Hoboken, Wiley, 2009, pp 257–323.
- 44 Halberg F, Sothorn RB, Cornelissen G, Czaplicki J: Chronomics, human time estimation, and aging. *Clin Interv Aging* 2008;3:749–760.
- 45 Halberg E, Halberg J, Halberg F, Sothorn RB, Levine H, Halberg F: Familial and individualized longitudinal autorhythmometry for 5 to 12 years and human age effects. *J Gerontol* 1981;36:31–33.
- 46 Halberg F, Gupta BD, Haus E, Halberg E, Dekker AC, Nelson W, Sothorn RB, Cornelissen G, Lee JK, Lakatua DJ, Scheving LE, Burns ER: Steps toward a cancer chronopolytherapy; in Acar J, Bastin R (eds): *XIV Congrès International de Thérapeutique*, 7–10 septembre 1977, Montpellier, France, sous la présidence de J Mirouze. Paris, Expansion Scientifique Française, 1977, pp 151–196.
- 47 Altman BJ, Hsieh AL, Sengupta A, Krishnanaiah SY, Stine ZE, Walton ZE, Gouw AM, Venkataraman A, Li B, Goraksha-Hicks P, Diskin SJ, Bellovin DI, Simon MC, Rathmell JC, Lazar MA, Maris JM, Felsner DW, Hogenesch JB, Weljie AM, Dang CV: MYC disrupts the circadian clock and metabolism in cancer cells. *Cell Metab* 2015;22:1009–1019.
- 48 Watanabe Y, Halberg F, Otsuka K, Cornelissen G: Toward a personalized chronotherapy of high blood pressure and a circadian overswing. *Clin Exp Hypertens* 2013;35:257–266.