Faculty of Medicine Masaryk University Brno Czech Republic

Department of Functional Diagnostics and Rehabilitation

PROCEEDINGS

SYMPOSIUM

CHRONOBIOLOGY IN MEDICINE

DEDICATED TO THE 85TH ANNIVERSARY OF PROFESSOR FRANZ HALBERG

Edited by: Cornélissen G., Kenner R., Fišer B., Siegelová J.

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The Symposium takes place under the auspices of

Prof. PhDr. Petr Fiala, Ph.D., Rector of Masaryk University Brno

Prof. MUDr. Jan Žaloudík, CSc., Dean of Faculty of Medicine Masaryk University Brno

MUDr. Michal Pohanka, Ph.D., Director of the St. Anna Teaching Hospital in Brno

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MAIN SPONSORS: Pliva - Lachema

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ISBN 80-7013-412-7

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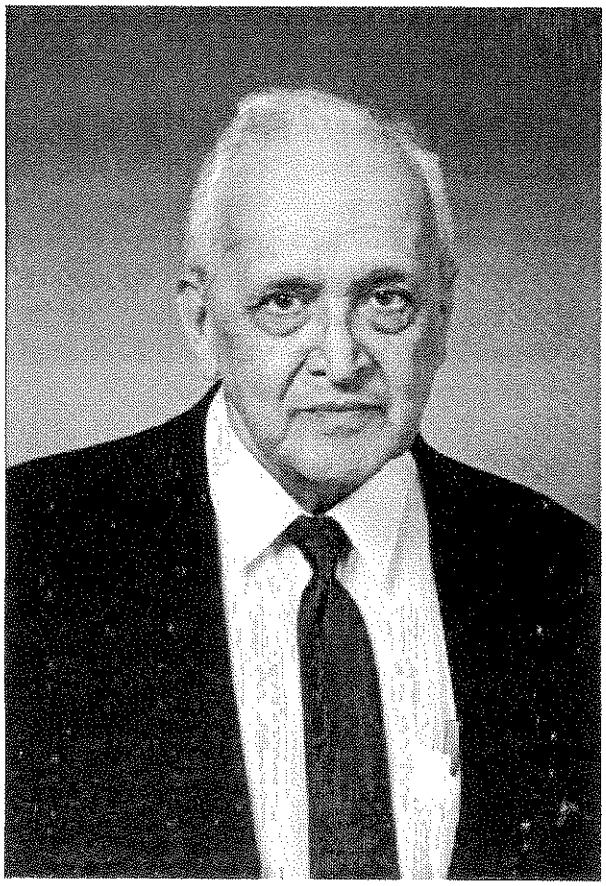


Franz Halberg, M.D., Dr. h.c. (Montpellier), Dr. h.c. (Ferrara), Dr. h.c. (Tyumen), Dr. h.c. (Brno), Professor of Laboratory Medicine and Pathology, Physiology, Biology, Bioengineering and Oral medicine, Director, Halberg Chronobiology Center, University of Minnesota, Mayo Mail Code 8609, Dept of Laboratory Medicine, Minneapolis Campus
420 Delaware Street SE, Minneapolis, MN 55455, USA

Prof. Dr. Franz Halberg

85 years of age

Prof. Halberg has dedicated almost 50 years of his life to chronobiological research. He is called the fater of chronobiology.

Chronobiology, the study of mechanisms underlying diversity in time, and chronomics, the mapping of chronomes - time structures, could complement genetics. It is the study of mechanisms underlying diversity in space and also genomics, the mapping of the genomes. Halberg made here focus on the chronobiologic-chronomic assessment of blood pressure and heart rate variability as the alternative to the spotcheck of the blood pressure advocated by official current guidelines. Chronobiology allow us to approach risks, diagnosis and treatment dependent on appointment time, especially of the dynamics of time, gender, age, ethnicity and geographical location.

His chronobiological studies represent a new original Minnesotan branch of science based upon resolving the chronome and its mapping from womb to tomb. Womb -to- tomb chronome initiative consists in extension of a unique existing data archive and reference standard bank on variables of biomedical interests: heart rate, blood pressure, body temperature, a host of chemical determinations on blood, saliva and urine.

Every biologic variable is characterized by chronomes, describing the structure of rhythms and trends in its physiological and pathophysiological range of variations. The chronome provides new endpoints for ruling in health or recognizing increased disease risk before the occurrence of overt illness. Prof. Halberg ´s long lasting basic scientific work is directed, at the beginning of the new century, to chronobiometry (physiological and statistical evaluation of the genetically anchored and cosmically influenced time structures), chronobioengineering (collecting physiological data by means of sophisticated equipment), chronobiological diagnosis of disease risk syndromes, and the chronotherapy, improvement of prognosis and treatment in different fields of medicine, and last but not least, chronoastrobiology focusing on rhythms and broader chronomes to explore the origins of life.

Needless to say, not only those researchers who are deeply interested in this field, but also others who are less interested, have all been strongly impressed by such incomparable records of work achieved by Prof. Halberg. In recent years he has been strenuously promoting chronobiological research further in the field of clinical medicine on the worldwide scale. We feel honored to have had the possibility of cooperation with Prof. Halberg since 1980s. In the year 2000, Prof. Franz Halberg from University of Minnnesota, USA, received the degree of honorary doctor of Masaryk University and thus have the honor also to be members of Masaryk University Brno. In the last 15 years, the cooperation between Masaryk University and University of Minnesota was intensive and was enlaged also to the international project BIOCOS.

The international project on The BIOsphere and the COSmos originated on June 30, 1997, when the Russian Academy of Medical Sciences convened a special session at its headquarters in Moscow to discuss and, at the end of this meeting, to unanimously endorse a project on "The BIOsphere and the COSmos" (BIOCOS).

The role of chronomics within the context of Non-communicable Diseases and Mental Health is mostly studied in cardiovascular variables but not limited only to the cardiovascular system. The same methodology remains applicable to a wide range of problems. Cancer prevention and optimization by the scheduling of treatment administration is another important problem. But the main focus of BIOCOS in health promotion upon the circulation by "prehabilitation", to reduce the cost of rehabilitation by education of use of chronobiology.

In 2004, Prof. Halberg is full of energy directed to discovering new laws of chronobiology. Ad multos annos!

Prof. MUDr. Jarmila Siegelová, DrSc.
Head, Dept. of Functional Diagnostics and Rehabilitation
St. Anna´s Teaching Hospital
Faculty of Medicine
Masaryk University
Pekařská 53
656 91 Brno

TIME STRUCTURES (CHRONOMES) IN US AND AROUND US: A TRIBUTE TO FRANZ HALBERG*

Germaine Cornélissen
Halberg Chronobiology Center
University of Minnesota, Mayo Mail Code 8609
420 Delaware St. S.E. Minneapolis, MN 55455, USA
TEL +1 612 624 6976 FAX +1 612 624 9989
E-MAIL corne001@umn.edu
Website: http://www.msi.umn.edu/~halberg/

*Update of "Chronobiology: a frontier in biology and medicine" published in Chronobiologia (16: 383-428, 1989) to honor Franz Halberg on the occasion of his 70th birthday.

La pensée ne doit jamais se soumettre,
Ni a un dogme, ni a un parti,
Ni a une passion, ni a un intéret,
Ni a une idée préconçue,
Ni a quoique ce soit,
Si ce n'est aux faits eux-memes,
Parce que pour elle, se soumettre,
Ce serait cesser d'etre.

Henri Poincaré

Support: Dr h.c. (mult.) Earl E Bakken Fund, University of Minnesota Supercomputing Institute

APPRECIATION

Gratitude is expressed to Jarmila Siegelova for organizing this symposium in honor of Franz Halberg, for being the pillar of BIOCOS in Europe, and for a professional lifetime of friendship which is deeply appreciated and cherished. It is fitting that Brno that saw the birth of genetics in the pea patch of Gregor Mendel is now embracing the development of chronomics, the mapping of broad time structures in us and around us. With Jarmila and her team, we learned about the changing time structure in early extrauterine life. We witnessed firsthand how the same low dose of aspirin was effective against blood clotting in the morning but not in early afternoon, and how this optimal time could differ with respect to the blood pressure lowering property of aspirin or the toxicity of its side effects to the gastrointestinal tract. Jarmila's current endeavors of 7-day/24-hour monitoring of blood pressure and heart rate establish new roadmaps. She aims with us in Minnesota at recognizing earliest rhythm alteration, as a warning of increased vascular disease risk. These harbingers can prompt the institution of prophylactic countermeasures, prehabilitation. This is a gift to Franz, who hopes that self-surveillance for risk detection and risk lowering will someday complement and possibly replace current spotcheck medicine based only on deviations from a broad homeostatic normal range.

Fifteen years ago, we celebrated the birth of a new science, chronobiology, in a special issue of the journal Chronobiologia, by honoring the 70th birthday of Franz Halberg [1]. While the discovery of important physiological problems and their mechanisms is certainly one of Franz's major achievements, the science itself would not have developed without his changing the very fundamentals of medicine and biology, by recognizing that variability and not homeostasis is the essence of life [2-6]. It is the privilege of only very few to have an impact in science, not only by original findings but primarily by a vision of their implications, that lead beyond a scientific breakthrough to a new way of thinking. By his deep sense of humility and his ability to sit in front of the facts without preconceived ideas, Franz has made a lasting dent in the history of medicine.

The purpose of this tribute is not to recount the early discoveries of the endogenicity of the circadian adrenal cortical cycle [2, 7-10], based on the free-running of circadian and other "circa" rhythms in (genetically) blind mice (born anophthalmic) [3, 11-13] and blinded mice (with surgery removing the transducer of light) [14], or in the absence of dominant environmental synchronizers such as the lighting regimen or mealtime, or a diet restricted in calories [4, 14, 15]. It is not to emphasize how the sequential phase relationship of circadian cellular rhythms, the labeling of phospholipid and the formation of hepatic RNA preceding that of DNA [12, 16] may shed light on life's origins, or to demonstrate the important clinical implications of the difference in outcome, life or death, from a variety of stimuli (noise, endotoxins or drugs) [17-20], or the rules of schedule shifts [4, 14, 16, 21-23]. It is not to enumerate each new rhythm added to the existing body of knowledge [4, 5, 24], or even to list multifrequency rhythms responsible for the chronomodulations [feedsidewards] that underlie the difference between the stimulation or inhibition of DNA synthesis [25] or cancer growth [26, 27] or the difference between life and death in response to the same dose of the same molecule [28]. Nor is it to document the early indirect evidence for an endogenous organismic time structure based on free-running [11; cf. also 9, 10, 13, 15], notably in objectively quantified isolation studies [29-31] and on work on human twins reared apart [5, 32], or to review the evidence for the synchronizing role of the socioecologic setting [12] that underlies the amenability of rhythms to change their temporal location in relation to environmental cycles, according

to specific rules of adjustment. An account of these early developments is reviewed elsewhere [179].

In 1989, we already noted that for chronobiology to evolve as a science, it was necessary to document the ubiquity of rhythms, to demonstrate their critical importance, and to develop the methods for their assessment and their interpretation [1, 4, 5]. With the help of several generations of chronobiologists worldwide who have in one way or another been associated with Franz Halberg and have contributed to what quantitative chronobiology is today, the pitfalls of a time-invariant physiology have been identified and the limiting view of homeostasis replaced by that of a partly built-in time structure (chronome) in health.

The purpose of this laudatio is to summarize the highlights of Franz's achievements during the last 15 years, thereby attempting to present a synthesis of the field Franz developed. Central to the new discipline is the concept of chronomes, broad time structures in us and around us, which Franz formulated explicitly following a session on chronobiology organized by the late Norberto Montalbetti at the XIV International Congress of Clinical Chemistry in San Francisco (July 22-26, 1990) [33, 34]. It led to the new field of chronomics [180], the mapping of chronomes, providing the needed foundation for future inroads in the dynamics of life.

It was tempting for Franz to cite Johnson [35] when he discovered the adrenal cortex to be indeed an "exceptionally substantial and durable self-winding and self-regulating physiological clock" [2]. But, as he accumulated data on a host of interacting rhythms with several frequencies, it rapidly became clear to Franz that organismic time structures are so much more than just "clocks". When rhythms (such as circaseptans) may have been acquired through evolution from cycles in the environment, and may still be influenced by them, "clocks" in the sense of time structures are much more than a timing system; they do not only adjust and even respond to the challenges of everyday life, but they also prepare us for them [2, 4]. For these reasons, Franz recognized early on the need for inferential statistical methods. He recognized time-measurement in biology as an important, but limited and potentially limiting concept (for instance, when the circaseptan aspects of a cancer treatment are ignored [36]).

Not only is hypothesis testing on more than one chronome component essential, so is parameter estimation. Visual inspection is insufficient to reliably appraise the information contained in a data set. By contrast, chronobiological analyses may resolve variability within the physiological range while also yielding information about any environmental influence and/or the endogenous nature of rhythms. It is no surprise that Franz introduced the periodogram [11, 37] and power spectrum [38, 39] into what became chronobiology, further developing a series of statistical procedures, of which the single cosinor [4, 40] is perhaps the most widely disseminated.

Among Franz's accomplishments during the past 15 years, apart from didactic endeavors with materials also posted on the Internet [41-45, 179; cf. also 46-49], three major avenues of research can be identified, namely cancer chronotherapy, chronocardiology and chronoastrobiology.

CANCER CHRONOTHERAPY

When, on December 27, 1990, Franz's wife, Erna, then 70 years of age, was diagnosed with a widely metastatic, undifferentiated (müllerian duct) adenocarcinoma involving the ovary, everything was placed on the back burner to shift the laboratory's focus to cancer

chronotherapy and how it could be optimized for the individual patient. As reviewed earlier [1], and elsewhere [50-54], with Erna's help, Franz had already worked extensively in two fields:

1. The chronobiologic N-of-1 studies, which in Franz's view are the essence of health care. In particular, two of his closest friends practiced extensive self-measurements, notably of blood pressure, since they were both MESOR-hypertensive: Frederic C. Bartter, of Bartter syndrome fame [55], head of the then-Endocrine-Hypertension Division and thereafter director of the Clinical Center at the U.S. National Institutes of Health (NIH), and Howard Levine, professor at the University of Connecticut and chief of medicine at the New Britain (Connecticut) General Hospital [56] (Franz served as a consultant to both institutions).
2. Cancer research, which in part led Franz to chronobiology [57]. In the experimental laboratory, cures not otherwise possible were achieved by Franz's chronotherapeutic designs of timed drug administration [58]. More importantly, 2-year disease-free survival was doubled for patients with perioral tumors when radiotherapy was administered at the time of peak tumor temperature [53, 59]. The usefulness of marker rhythmometry as a guide for treatment timing and for assessing the patient's response to treatment was demonstrated.

Erna, who with Franz had introduced autorhythmometry at home, among friends, and in schools [60, 61], was more than willing to collect blood, urine and saliva samples throughout her illness, while also wearing an ambulatory blood pressure and heart rate monitor, with occasional additional self-measurements or self-ratings (e.g., of mood). Her active participation in research related to her own treatment, most of it published [62; cf. also 63-78], in the opinion of her oncologist, contributed to her 3-year survival [69]. During these 3 years, much new information was gathered. First and foremost, emphasis was placed on timing to optimize treatment effectiveness first while also attempting to minimize toxicity [41]. This was made possible to a large extent by the availability of tumor markers assessable non-invasively in urine and/or saliva [70, 71], where they can undergo large and statistically predictable changes [62]. Methods applicable to the individual patient were badly needed and were forthcoming. Designs for "phase-zero" trials were formulated and their statistical power assessed and compared with conventional approaches [72-74]. Too late for Erna's sake, the control charts of Hawkins' self-starting cumulative sum [75, 76] later found applications in optimizing the timing of anti-hypertensive medication [42, 77].

At the very end of a long battle with cancer, Erna's last contribution, named after her (the Erna-test) [78], combined chemosensitivity assays in vitro [79] with marker rhythmometry for targetting cancer treatment both in time and in kind. Erna demonstrated, in the very last days of her life, just how critical timing can be: a depression of the tumor marker used (urinary gonadotropin peptide) was observed when 5-FU, identified by a ChemoResponse Assay to show in vitro antineoplastic activity in her ascitic fluid, was administered in the late evening (at 22:00), but not in the morning or in the afternoon [67, 78]. Remarkably, this timing is in keeping with the recommended infusion schedule of the drug (with maximal dosing by night), based on a subsequent large clinical trial [80], a result supporting the ultimately indispensable N-of-1 chronobiologic design, that gains greatly when it relies on time series collected and analyzed chronobiologically in lieu of spotchecks on rollercoasters. The assessment of statistically significant changes

in rhythm characteristics of the given patient in relation to risk elevation and/or as a response to treatment is then feasible and should become indispensable in routine care.

The non-invasively sampled marker rhythms of large amplitude in CA125, CA130, UGP, M-CSF and OVX1 in saliva and urine are a testimony of Erna's discipline and devotion to science. Some of these marker rhythms were in their stage of development at the time of her diagnosis. Erna's legacy is her careful mapping of their time structure and of their promise to help guide the timing of chemotherapy for an optimization of treatment efficacy first and foremost. On September 8, 1993, the day she died, the world lost not only a conscientious researcher but a devoted friend, and to me a mother-away-from-home. On her request, her ashes were scattered in the Mississippi River, but her spirit remains very much with us, ever present in her kind and gentle way to guide the research by the extensive data bases she amassed on herself unselfishly to help others.

Her data remain a priceless source of information to which we can turn to whenever the need arises. Recently, her data helped solve a question raised in evaluating a thesis from Armenia. The thesis reported a 12-hour component in melatonin. We were testing the hypothesis that there was a secondary peak in melatonin production in the afternoon, intimated, for instance, by a re-analysis of salivary data from Vollrath [81]. We could not obtain an answer by numerical analyses alone (aimed at demonstrating a secondary peak in the afternoon; the statistical significance of the 12-hour harmonic could merely reflect the non-sinusoidality of the circadian rhythm in melatonin). Erna's abundant urine collections provided at least a tentative answer in the affirmative to the question whether there can be a peak in the afternoon that may be physiologically independent of the nightly peak. For a while, at a time when the disease had progressed beyond the hope of recovery, Erna's pattern in urinary melatonin concentration was characterized by a single prominent peak in the afternoon, in the absence of the nocturnal elevation usually found for melatonin in her and almost all others. The disease had shown what numerical analyses could not resolve: at least for a while, the nocturnal peak was removed. Thank you so much, Erna! (We also wish to thank Robert C. Bast Jr., whom we had never met until that time, and who upon our inquiry asked whether 100 free determinations of CA125, the marker he had discovered, were enough as a start. Thereafter, he did very many more determinations, also on additional markers. Our thanks are also addressed to Kohji Tamura, who called the Daiichi company, which donated $50,000 worth of CA130 kits to help Erna. We thank Roger Walker, who helped with the determinations of UGP, and of course Erhard Haus, who eventually took charge of the laboratory work, including the determinations of melatonin. We are greatly indebted to all of them, and many others. We are indebted to RK Singh and his team for resuming marker rhythm-guided cancer chronotherapy in Lucknow, India, and to Zhengrong Wang for attempting to do the same in China [181].)

CHRONOCARDIOLOGY

Through the generosity of Masayuki Shinoda, then President of Colin Electronics (Komaki, Japan), extended to Franz and his team, large archives of ambulatory blood pressure (BP) and heart rate (HR) profiles accumulated. They provided strong evidence for the need to account for day-to-day changes in these variables in the same way a circadian assessment considers the hour-to-hour variability. The evidence led to the recommendation of around-the-clock monitoring for 7 days at the outset [182, 183], to be

continued whenever needed, until monitoring for a lifetime becomes more readily feasible, a goal pursed by the Phoenix group (http://www.phoenix.tc-ieee.org/).

By 1988, major findings had been summarized in a volume of annotated illustrations [82]. Methodology had developed concomitantly under Franz's leadership. In particular, the "sphygmochron" [83] was introduced. We were favored by the help of Patrick Delmore, Head of Communications at Medtronic, and Earl E. Bakken, the founder of that company, the builder of the first implanted pacemaker for long-term use, a chronobiologist in his own right, who with Franz introduced the concept of a free-running rhythm half a century ago.

The sphygmochron is a computer summary of results from chronobiological analyses performed on BP and HR data collected around the clock, preferably by ambulatory monitoring. It consists of two approaches, one parametric (model-dependent), the other non-parametric (model-independent). The parametric approach entails the least-squares fit of a two-component model consisting of cosine curves with periods of 24 and 12 hours. Estimates are obtained for the MESOR (midline-estimating statistic of rhythm), a rhythm-adjusted mean, and for the amplitude and (acro)phase of each component, measures of (half) the extent of predictable change within a cycle, and of the timing of overall high values recurring in each cycle, respectively.

To circumvent any limitations of the parametric approach based on a model that may not invariably suffice to appropriately describe the circadian pattern, the analyses include a nonparametric approach, based on stacking the data over an idealized cycle. It was deemed desirable since it was readily understood and could serve as a guide for timing treatment. It can serve as a complement to adjusting the model by the choice of different harmonic terms for each profile. Despite the merits of improving each individual fit, it was felt important to standardize the model, so that reference values could be derived for each parameter of the model. The given subject's rhythm characteristics could then readily be compared with those of peers. Values outside the norms could then become putative risk indicators.

In the nonparametric approach, the subject's data are compared by computer to the time-varying upper 95% prediction limit (chronodesm) of clinically healthy peers, matched by gender, age and ethnicity. Instead of asking only whether a BP value is too high or acceptable, this approach answers more complete and often more pertinent questions, such as "For what percentage of the 24-hour day are the subject's BP readings above the chronodesmic limit?", "By how much is the BP excessive ?" (gauged by the "area under the curve", delineated by the subject's profile when it exceeds the limit and the limit itself; expressed in mm Hg x hour during 24 hours), and "When does most of the excess occur ?". Years later, part of this "hyperbaric index" (HBI) concept was copied to assess a BP load, with the shortcomings, however, that the BP load considers only the percentage time elevation and not the "area under the curve", and that it uses time , gender , age , and ethnicity-unqualified fixed limits as reference [82, 83]. Elsewhere, the advantages of the HBI over the BP load are illustrated by a comparison of patients seen at the Mayo Clinic who have very similar BP loads but drastically different HBIs [84].

In addition to the time-specified reference limits for the interpretation of time-measurements, reference limits were also derived for the interpretation of the circadian rhythm characteristics. If the BP MESOR exceeds the upper 95% prediction limit, MESOR-hypertension is diagnosed; if the circadian amplitude is excessive, CHAT (circadian hyper-amplitude-tension) is diagnosed; and if the circadian acrophase occurs at an odd time, circadian ecphasia is diagnosed.

Analyses of long time series [85-92, 182, 183] yielded added evidence for the need to monitor for spans longer than 24 hours. The extent of day-to-day variability can be so large for some individuals that a diagnosis based on a single 24-hour span can be very misleading. At a meeting of the International Society for Research on Civilization Diseases and the Environment (New SIRMCE Confederation) in Brussels, Belgium, on March 17-18, 1995, a resolution was reached that recommended monitoring for 7 days at the outset [42]. Not only does the circadian assessment become more reliable, a glimpse if not insight into the about-weekly (circaseptan) component is also obtained. Circaseptans were found to be particularly prominent in early extrauterine life [93] and to regain prominence in the elderly [94], while persisting in isolation from society [95, 96].

The resolution reached in Brussels [42] also made provisions for the handling of large volumes of data by Franz's introduction of three easy steps: windowing, compacting, and recycling [97]. Instead of being discarded, the data are saved for as-one-goes analyses in a form compacted into characteristics of the rhythms with the next higher frequency, resolved by repeated passes directly from the original data, as long as data storage permits it, or from the computed endpoints of chronome elements such as rhythm characteristics of already-resolved higher frequency components, when compacting becomes indispensable [95, 97]. The "recycling" by these repeated passes of accumulating BP and HR data led to the identification of new disease risks, such as CHAT, CAHRVs (chronome alterations of heart rate variability - e.g., a decreased heart rate variability, DHRV, along the circadian scale), and an excessive pulse pressure (EPP). These conditions relate to altered variability within the physiological range, which, once recognized, can prompt the institution of preventive measures.

Table 1 provides a chronological account of the several milestones in Franz's laboratory that led to the identification of CHAT, CAHRVs, and EPP as disease risks. With or without the MESOR, the circadian BP amplitude was found to be a predictor of vascular disease risk and mortality [98, 184-186]. Retrospective analyses confirmed the results by showing that an excessive circadian BP amplitude is associated with an elevated left ventricular mass index, determined on all subjects, and used as a surrogate outcome measure [99-101].

In a 6-year prospective study on 297 patients generously provided by Kuniaki Otsuka, among the risks assessed concomitantly, CHAT was found to represent the largest (720%) increase in the risk of cerebral ischemic events, compared with 310%, 370%, 160%, 170% and 150% in relation to MESOR-hypertension, old age, a positive family history of high BP and/or related vascular disease, smoking and alcohol consumption, respectively. A reduced 24-hour standard deviation (SD) of HR (DHRV) was also associated with an increased risk of vascular morbidity, coronary artery disease and cerebral ischemic events in particular [102]. In both cases, the endpoint exhibits a nonlinear relation with vascular disease risk [103].

The lack of a statistically significant correlation between the circadian BP amplitude and the 24-hour SD of HR suggested that CHAT and DHRV constitute separate disease risks. The likelihood of a morbid event is about doubled when both conditions are present than when either diagnosis is present alone [104]. EPP is also mostly a risk factor separate from CHAT and/or DHRV (Figure 1 and Table 2). The relative risk of a morbid event is calculated by comparing patients who had one or several chronome alterations with patients who had acceptable dynamics (circadian BP amplitude, 24-hour SD of HR, and pulse pressure).

To detect CHAT, and for other diagnostic and therapeutic reasons, Franz has advocated that single measurements should be replaced by an around-the-clock profile, for a week or longer if need be, at the outset, to be obtained preferably by ambulatory monitoring [105]. Indeed, several studies comparing the diagnosis reached on the basis of ambulatory monitoring (for 7 days) versus single measurements taken in the physician's office, reached the conclusion that an error of about 40% may be associated with the current approach. Two culprits were identified, namely:

1. The reliance on single time-unspecified measurements, based on the mistaken assumption that a procedure used on thousands of people in large clinical trials can readily be transferred, without qualification, for a decision to be made for the individual patient. Even the most accurate measurement taken in the clinic, usually associated with an error of less than 5 mm Hg, needs to be interpreted in the light of a within-hour SD of BP of the order of 7 mm Hg and a within-day change often exceeding 50 mm Hg; even a 24-hour profile obtained by ambulatory monitoring is no gold standard in view of the large day-to-day changes in circadian characteristics [182, 183]; and
2. The anachronism of interpreting measurements of a variable known to undergo a prominent circadian rhythm in the light of fixed limits, irrespective of time, age, or gender.

From a purely theoretical viewpoint, a sizeable number of patients with a circadian amplitude close to the upper limit of acceptability and a commonly found acrophase in mid-afternoon, is likely to be diagnosed as normotensive in the morning but as hypertensive in the afternoon [106]. This situation was actually encountered in practice, as described in print in detail by Frederic C. Bartter [107], prompting him to emphasize the indispensability of the cosinor in routine practice.

The week-long monitoring profile also serves as a guide for timing treatment administration if need be. Results [42] indicate that certain antihypertensive drugs can lower an excessive circadian BP amplitude. For instance, in the subjects investigated, captopril retard and atenolol slightly reduce the circadian amplitude of diastolic BP but not that of systolic BP, whereas a long-acting formulation of carteolol reduces the circadian amplitude of both systolic and diastolic BP in the majority of patients, albeit not in all of them [108, 187]. The timing of treatment administration can also affect the circadian waveform of BP, and influence outcome, such as the incidence of strokes and overall cardiovascular disease [188]. For those patients diagnosed with CHAT who are otherwise MESOR-normotensive, the first choice of treatment may be non-pharmacological. In this case, autogenic training, a relaxation technique, can lower the circadian BP amplitude in some patients [109-111].

As noted by Franz, focus on alterations in pattern (or variability) requires a change in attitude akin to that needed for the case of universal literacy [112]. The association of CHAT and CAHRVs with catastrophic disease renders the assessment of variability into a community concern. Franz rightly notes that we all pay, indirectly if not directly, for the care of massive strokes, myocardial infarctions or nephropathy, apart from the immeasurable suffering and disability of those afflicted. In Minnesota, Dan Wall -- an attorney and past mayor of Roseville, a suburban city of about 35,000 residents -- succeeded in acquiring for his constituents ten automatic ambulatory instruments for the week-long profiles of around-the-clock monitoring of BP and HR. The instrumentation from A&D (Tokyo, Japan) is generously offered at a greatly reduced price by the company

for all those working on stroke prevention with Franz's group. A public service task of physiological monitoring should indeed be within the mandate of those not only in local, but also in federal government agencies dispensing resources, and it is just a first step toward education in a much broader chronobiologic literacy [112]. The healthwatch conceived for Roseville was subsequently implemented in Urausu, Hokkaido, Japan, by Kuniaki Otsuka. The 7-day/24-hour monitoring is currently ongoing in the department of functional physiology in Santa Anna Hospital, Brno, Czech Republic, under the guidance of Jarmila Siegelova and Pavel Homolka, in Moradabad, Amritsar, and Lucknow, India, implemented by RB Singh, Adarsh Kumar, and RK Singh, respectively, and in the general practice of two Belgian physicians, among other collaboration in Armenia, Italy, Mexico, Norway, the People's Republic of China, as well as in California, Michigan and Minnesota [183].

CHRONOASTROBIOLOGY

Since the definition of "circadian" [3], Franz has coined many new terms, as the need arose, to introduce new concepts in a new science. He defines "chronoastrobiology" as a term derived from "chronome" (time structure) and "astrobiology" [49]. Chronoastrobiology, including chronoastronautics, is a branch of biology concerned with transdisciplinary methods for the coordinated mapping of interdigitated and intermodulating time structures (chronomes) in us and around us. The mapping of feedsideward interactions among chronomes for the derivation of reference standards quantifies the current temporal organization of physiological functions. Photic and other environmental influences upon it bear upon an understanding of the sites of life's origins and of worlds before ours, while underlying a preventive health care needed in travels away from hospitals, on earth and in space.

Toward this goal, Franz started a coordinated comparative physiological and physical (and, when pertinent, archival) monitoring and analyses, known as the project on the BIOsphere and the COSmos (BIOCOS) [113]. As reported above, this international endeavor has already borne fruit with the identification of disease risks, as a step toward risk lowering on earth, as a model also for use during travel in extraterrestrial space. Work in this direction led to the recognition of a reciprocity between time structures in us and around us [189-195].

The monitoring in different geographic locations of chronomes of selected variables during human ontogeny is being carried out in the context of a comparative physiology, focusing also on cosmo-helio-seleno-geophysical time structures that may have existed at the sites of life's origins, wherever they may have been. The analysis of data on "living fossils" may shed new light about how the organismic structures evolved not only in space (classical morphology), but also in time (chronobiology as a morphology in time). For this purpose, physical changes, as events or continuous records, monitored concomitantly, are being analyzed for matches or near-matches between geophysical features and biological ones, on earth if not yet in space, retrospectively as well as prospectively.

A finding reported at a meeting in Tokyo on Nov. 11, 2000 [114], relates to the ubiquity of about 10.5-year cycles similar to the solar activity cycle, suggesting that environmental influences consist not only of photic, but also of non-photic solar effects, mediated perhaps, at least in part, via geomagnetic disturbance. Franz's reasoning is that rhythmic elements of biological chronomes with a common genetic origin (in the sense of a classical homology) may also have a common environmental cycle, responsible for their genetic coding to start

with. Franz postulates a broad physico-biological homology, as compared to the classical homology implying only a common genetic origin, which he extends to the organismic make-up in time, as done conventionally, albeit somewhat controversially, with respect to a spatial morphology. In this framework, a physical near-match in the environment may be sought for each built-in biological rhythm, and vice versa. This rule is now extended successfully to components with periods slightly, but statistically significantly longer and/or shorter than precisely one year, the "transyears" and the "cisyears". Most interesting is the recent finding of such spectral components in the horizontal component of the interplanetary induction vector (Figure 2).

Also of interest in the context of magnetoperiodism is the biological week [115], free-running from the social week [196]. Franz found a weak near-match of about 6.75 days in a 59-year record of the geomagnetic index, Kp [116], later confirmed by physicists [117, 118, 197]. The near 7-day environmental counterpart's weakness is in keeping with a prominent about-weekly biological component that may be in part the result of an integrative evolution: in their genesis Away from the overpowering effect of a daily alternation of light and darkness, requirements internal to an organism may have prevailed, to lock into some other signal, whatever its intensity may be. The weak about 7-day geomagnetic component happened to be nearest to internal needs, such as those for growth and repair, by processes that took several days. This is the more plausible since a theoretical biochemical basis for circaseptans has also been proposed [119, 120].

Circaseptans are found prominently in earliest extrauterine life in humans, when they are synchronized by the single stimulus of birth that carries no environmental 7-day information [93, 191]. They are also observed early in the extrauterine life of rats [121] and pigs [122]. Like humans, rats and pigs lose the prominence of the biological week in early stages of extrauterine ontogeny. By contrast, a circaseptan component is dominant in the locomotor activity of crayfish at 6 months of age [123], a result suggesting that certain multicellular organisms may be better "fossils" for specific evolutionary information, as compared to other species [124]. Circaseptans are a feature of immunity as well. They are observed, for instance, in the distribution of kidney (and other) graft rejection episodes, characterizing patients operated on different days of the week in hospitals in Minnesota, Paris and Milan [125], or in the experimental animal laboratory. Meta-analyses quantify them in the compensatory hypertrophy after unilateral nephrectomy or contralateral ischemia [41, 126, 127]. The optimization of the timed administration of immunomodulators could gain greatly from consideration of the circaseptan as well as the circadian component [36]. The pineal in particular is characterized by prominent circaseptans [5, 128, 129]. Amplification of circaseptans has also been observed after lesioning of the suprachiasmatic nuclei [199,200].

Circaseptans and circasemiseptans are also found in early phylogeny in eukaryotic unicells and prokaryotes [41, 124, 128-132, 191], including bacteria [133]. Evidence for a genetically anchored, environmentally resonant biological week and half-week is provided by the results of Syutkina et al. [134], showing that the (nonlinearly-assessed) circaseptan period of neonatal BP and HR correlates with that of the local geomagnetic disturbance index K recorded during matching spans, as well as by the amplification of circaseptans in human HR by solar circaseptans [135]. That these components are not trivial is illustrated by the non-random circaseptan patterns in morbidity and/or mortality from various cardiovascular causes, notably myocardial infarctions and strokes [116, 136, 137].

An increase in the incidence of myocardial infarctions in association with magnetic storms, reported by several investigators from Russia [138, 139], Israel [140] and Mexico

[141], accounts in Minnesota for a 5% (220 cases/year) increase in morbidity during years of maximal solar activity by comparison with years of minimal solar activity [48, 49, 142, 190]. Magnetic storms are also found to decrease HR variability [48, 49, 143, 144, 190], indicating a possible mechanism, since a reduced HR variability is a prognostic factor for coronary artery disease and myocardial infarction [145-148]. With Kuniaki Otsuka's Asian, now International Chronome Ecological Study of Heart Rate Variability, which joined forces with BIOCOS, longitudinal ECG records for one week or longer spans [149] were collected in different geographic locations, notably in the auroral oval, where magnetic storms are stronger and more frequent [150].

Melatonin has been invoked as a possible mediator of magnetic effects [151-156]. The pineal, a site where melatonin is produced, is apparently capable of responding to variations of the order of a few nT induced by the solar wind via the magnetosphere [157-160]. Of interest is the report of a decreased melatonin synthesis in patients with coronary artery disease [161]. Urinary melatonin excretion had also been found to correlate with cardiovascular disease risk [162].

Non-photic solar cycles are likely to be reflected in the time structure of melatonin. Tarquini et al. [163] in Florence, Italy (43.47°N) reported that circulating melatonin during the daytime undergoes circannual changes, whereas by night, when circulating melatonin concentrations are high, an about half-yearly pattern predominates. The pattern is reminiscent of the variation in the geomagnetic index Kp, also characterized by a very stable circasemiannual variation, with a large amplitude, peaking at the equinoxes [164, 165]. Based on longitudinal monthly data collected (around noon) over one year in Oulu, Finland (65.00°N), Martikainen et al. [166] reported a prominent half-yearly component in plasma melatonin. Randall [167] interprets the half-yearly signature by emphasizing that at high latitudes, the corpuscular radiation enters the atmosphere and magnetic disturbances induced by the solar wind, also characterized by a stable half-yearly cycle, are more pronounced [167]. An about half-yearly pattern was also observed in another study of night-time urinary melatonin excretion by 16 healthy volunteers followed longitudinally for one year, with peak values observed in June and November [168; cf. 169]. A latitudinal dependence was reported for the mean melatonin concentrations of overnight urine samples collected each month for 12 to 16 months from 321 healthy subjects at 19 medical centers in 14 countries distributed on 5 continents [170].

CONCLUDING REMARKS

Half a century ago, the birth of chronobiology as a science sui generis came about to a large extent due to Franz's demonstration of free-running. In mice, in the absence of the eyes, variables ranging from core temperature to hepatic glycogen content and serum corticosterone continued to cycle with a period close to but statistically significantly different from 24 hours. The circadian period differed among animals and among different variables in the same individual. This finding provided a basis for the endogenicity of rhythms in general and for the adrenal cycle in particular. Today, the genetic basis of circadian rhythms is no longer disputed [for review see 171]. Thus, fos family genes have been reported to exhibit differences in their specific expression patterns in the suprachiasmatic nuclei; photic and intrinsic circadian coordination may reside in separate cell populations in the ventrolateral and dorsomedial subdivisions [172], allowing for cell specificity of their respective circadian function(s).

While the vast literature on the molecular genetic basis of rhythms focuses almost exclusively on the details of the circadian system, a few studies suggest a molecular basis for ultradian and infradian rhythms as well. Konopka et al. [173] in particular have followed upon earlier work by Kyriacou and Hall [174], revealing that the period gene in *Drosophila melanogaster* coordinates not only circadian rhythms associated with adult emergence and behavior, but also a much higher frequency rhythm that accompanies the male's courtship song. About 1-min oscillations in the rate of sound production reportedly, in turn, are sped up, slowed down, or seemingly eliminated in three "per" mutants, an extension from "clock" to "chronome". It has also been suggested that infradians may emerge from a weak coupling of a circadian "clock" gene with its environment [198].

That infradian components also have a molecular genetic basis is likely from work on plants, when built-in photoperiodic aspects of responses in plants include flowering that is day-length-dependent [175-177]. Work on *C. elegans* further analyzes a molecular genetic basis to embryonic development possibly related chemically to the circadian system, while the heterochronic gene lin 42 oscillates apparently with an ultradian (reportedly about 6-hour) component [178]. Franz's work on a broader-than-circadian time structure, that includes trends with age as well as disease risk, is thereby vindicated at the molecular level. Just as circadians were described as "paranoia" when Franz coined the term but have become fashionable today, the concept of chronomes (much broader than clocks) is bound to prevail if concern for them leads to the difference between delaying rather than accelerating malignant growth [36].

Nearly half a century after showing the effect of light upon rhythms at different levels of organization, Franz's insight has led him back to realize the importance of understanding and assessing also non-photic environmental influences on biota. Still a controversial topic, it may nonetheless prove to be a critical one, primarily at a time when humans are getting ready for long journeys into space, away from hospitals. Any untoward effects from non-photic solar effects, perhaps on individuals who may be more susceptible at the outset by virtue of alterations in the variability of their BP or HR within the physiological range, warrant immediate attention so that countermeasures may be applied preventively.

Franz's vision, combined with his incredible persistence and intellectual clarity in the face of entrenched thinking, which established procedures and fields that transcend disciplinary boundaries, sets him apart as an extraordinary human being and a truly great scientist. It has truly been an honor and a privilege to have him as a mentor and as a guide through life, things for which I shall eternally be grateful. From the bottom of my heart, I do wish you

MANY HAPPY RETURNS!

REFERENCES

1. Cornélissen G, Halberg E, Halberg Francine, et al. Chronobiology: a frontier in biology and medicine. Chronobiologia 16: 383-408, 1989.
2. Halberg F. Some physiological and clinical aspects of 24-hour periodicity. J-Lancet (Minneapolis) 73: 20-32, 1953.
3. Halberg F. Physiologic 24-hour periodicity; general and procedural considerations with reference to the adrenal cycle. Z Vitamin-, Hormon- u Fermentforsch 10, 225-296, 1959.
4. Halberg F. Chronobiology. Ann Rev Physiol 31: 675-725, 1969.
5. Halberg F. *Quo vadis* basic and clinical chronobiology: promise for health maintenance. Am J Anat 168: 543-594, 1983.
6. Halberg F, Cornélissen G, Bakken E. Caregiving merged with chronobiologic outcome assessment, research and education in health maintenance organizations (HMOs). Progress in Clinical and Biological Research 341B: 491-549, 1990.
7. Halberg F, Visscher MB, Flink EB, Berge K, Bock F. Diurnal rhythmic changes in blood eosinophil levels in health and in certain diseases. J-Lancet (Minneapolis) 71: 312-319, 1951.
8. Halberg F, Flink EB, Visscher MB. Alteration in diurnal rhythm in circulating eosinophil level in adrenal insufficiency. Am J Physiol 167: 791, 1951.
9. Halberg F, Howard RB. 24-hour periodicity and experimental medicine. Example and interpretations. Postgrad Med 24, 349-358, 1958.
10. Halberg F, Engeli M, Hamburger C, Hillman D. Spectral resolution of low-frequency, small-amplitude rhythms in excreted 17-ketosteroid; probable androgen induced circaseptan desychronization. Acta endocrinol (Kbh) Suppl 103, 5-54, 1965.
11. Halberg F. Beobachtungen über 24 Stunden-Periodik in standardisierter Versuchsanordnung vor und nach Epinephrektomie und bilateraler optischer Enukleation, 20th meeting of the German Physiological Society, Homburg/Saar, September, 1953. Ber ges Physiol 162: 354-355, 1954.
12. Halberg F, Halberg E, Barnum CP, Bittner JJ. Physiologic 24-hour periodicity in human beings and mice, the lighting regimen and daily routine. In: Photoperiodism and Related Phenomena in Plants and Animals (Withrow RB, ed.), Ed. Publ. No. 55, Am Assn Adv Sci, Washington, DC, 1959, pp. 803-878.
13. Halberg F. Body temperature, circadian rhythms and the eye. In: La Photorégulation de la Reproduction chez les Oiseaux et les Mammiferes (Benoit J, Assenmacher I, eds). Paris, CNRS #172, 1970, pp. 497-528. Discussion remarks pp. 520-528. (See additional discussion remarks on pp. 47, 51, 67, 69, 90, 113-116, 164-165, 187, 209, 380, 381, 382-383, 384, 406, 407, 408, 541, 542, 546.)
14. Halberg F, Visscher MB, Bittner JJ. Relation of visual factors to eosinophil rhythm in mice. Amer J Physiol 179: 229-235, 1954.
15. Sundararaj BI, Vasal S, Halberg F. Circannual rhythmic ovarian recrudescence in the catfish *Heteropneustes fossilis* (Bloch). In: Toward Chronopharmacology, Proc. 8th IUPHAR Cong. and Sat. Symposia, Nagasaki, July 27-28, 1981 (Takahashi R, Halberg F, Walker C, eds). Oxford/New York, Pergamon Press, 1982, pp. 319-337.
16. Halberg F, Barnum CP, Silber RH, Bittner JJ. 24-hour rhythms at several levels of integration in mice on different lighting regimens. Proc Soc exp Biol (NY) 97, 897-900, 1958.

17. 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 (NY) 88: 169-173, 1955.
18. Halberg F. Symposium on "Some current research methods and results with special reference to the central nervous system." Physiopathologic approach. Amer J ment Defic 65, 156-171, 1960.
19. Halberg F, Johnson EA, Brown BW, Bittner JJ. Susceptibility rhythm to E. coli endotoxin and bioassay. Proc Soc exp Biol (NY) 103, 142-144, 1960.
20. Halberg F. Grundlagenforschung zur Aetiologie des Karzinoms. Mkurse ärztl Forbild 14, 67-77, 1964.
21. Halberg F, Jacobson E, Wadsworth G, Bittner JJ. Audiogenic abnormality spectra, 24-hour periodicity and lighting. Science 128, 657-658, 1958.
22. Halberg F, Albrecht PG, Barnum CP Jr. Phase shifting of liver-glycogen rhythm in intact mice. Amer J Physiol 199, 400-402, 1960.
23. Halberg F. Organisms as circadian systems; temporal analysis of their physiologic and pathologic reponses, including injury and death. In: Symposium on Medical Aspects of Stress in the Military Climate, Walter Reed Army Institute of Research (Col. William D. Tigertt, Medical Corps, Director and Commandant), Walter Reed Army Medical Center (Maj. Gen. A.L. Tynes, Medical Corps, Commanding), Washington DC, 22-24 April 1964. U.S. Government Printing Office: 1965-778-714, pp. 1-36.
24. Halberg F, Lagoguey JM, Reinberg A. Human circannual rhythms in a broad spectral structure. Int J Chronobiol 8: 225-268, 1983.
25. Walker WV, Russell JE, Simmons DJ, Scheving LE, Cornélissen G, Halberg F. Effect of an adrenocorticotropin analogue, ACTH 1-17, on DNA synthesis in murine metaphyseal bone. Biochem Pharmacol 34: 1191-1196, 1985.
26. Halberg F, Cornélissen G, Conti C, et al. The pineal gland and chronobiologic history: mind and spirit as feedsidewards in time structures for prehabilitation. In: The Pineal Gland and Cancer: Neuroimmunoendocrine Mechanisms in Malignancy (Bartsch C, Bartsch H, Blask DE, Cardinali DP, Hrushesky WJM, Mecke W, eds). Heidelberg: Springer, 2000, pp 66-116.
27. Cornélissen G, Halberg F, Perfetto F, Tarquini R, Maggioni C, Wetterberg L. Melatonin involvement in cancer: methodological considerations. In: Bartsch C, Bartsch H, Blask DE, Cardinali DP, Hrushesky WJM, Mecke W, editors. The Pineal Gland and Cancer: Neuroimmunoendocrine Mechanisms in Malignancy. Heidelberg: Springer, 2000: 117-149.
28. Halberg F. Chronobiologic engineering. In: Infusion Systems in Medicine (Ensminger WD, Selam JL, eds). Mt. Kisco, NY: Futura Publishing Co, 1987, pp. 263-297.
29. Reinberg A, Halberg F, Ghata J, Siffre M. Spectre thermique (rythmes de la température rectale) d'une femme adulte avant, pendant et apres son isolement souterrain de trois mois. CR Acad Sci (Paris) 262, 782-785, 1966.
30. Siffre M, Reinberg A, Halberg F, Ghata J, Perdriel G, Slind R. L'isolement souterrain prolongé. Etude de deux sujets adultes sains avant, pendant et apres cet isolement. Presse méd 74, 915-919, 1966.
31. Apfelbaum M, Reinberg A, Nillus P, Halberg F. Rythmes circadiens de l'alternance veille-sommeil pendant l'isolement souterrain de sept jeunes femmes. Presse méd 77: 879-882, 1969.
32. Hanson BR, Halberg F, Tuna N, Bouchard TJ Jr, Lykken DT, Cornélissen G, Heston LL. Rhythmometry reveals heritability of circadian characteristics of heart rate of human twins reared apart. Cardiologia 29: 267-282, 1984.

33. Cornélissen G, Halberg F, Montalbetti N, et al. Clinical chemistry chronobiometry improves averages as MESORs, transforming confusing variation into new, sensitive, useful parameters. Clin Chem 36: 930-931, 1990.
34. Halberg F, Hermida R, Cornélissen G, et al. Chronobiologic and chrono-pharmacologic risk assessment, diagnosis and treatment monitoring. Clin Chem 36: 932-933, 1990.
35. Johnson MS. Effect of continuous light on periodic spontaneous activity of white-footed mice (*Peromyscus*). J Exp Zool 82: 315-328, 1939.
36. Halberg E, Halberg F. Chronobiologic study design in everyday life, clinic and laboratory. Chronobiologia 7: 95-120, 1980.
37. Koehler F, Okano FK, Elveback LR, Halberg F, Bittner JJ. Periodograms for the study of physiologic daily periodicity in mice and in man. Exp Med Surg 14, 5-30, 1956.
38. Halberg F, Panofsky H. I. Thermo-variance spectra; method and clinical illustrations. Exp Med Surg 19, 284-309, 1961.
39. Panofsky H, Halberg F. II. Thermo-variance spectra; simplified computational example and other methodology. Exp Med Surg 19, 323-338, 1961.
40. Halberg F, Tong YL, Johnson EA. Circadian system phase-an aspect of temporal morphology; procedures and illustrative examples. Proc. International Congress of Anatomists. In: The Cellular Aspects of Biorhythms, Symposium on Biorhythms, Mayersbach H.v. ed., New York: Springer-Verlag, 1967, pp. 20-48.
41. Cornélissen G, Halberg F. Introduction to Chronobiology. Medtronic Chronobiology Seminar #7, April 1994, 52 pp. (Library of Congress Catalog Card #94-060580; http://www.msi.umn.edu/~halberg/)
42. Halberg F, Cornélissen G, International Womb-to-Tomb Chronome Initiative Group: Resolution from a meeting of the International Society for Research on Civilization Diseases and the Environment (New SIRMCE Confederation), Brussels, Belgium, March 17-18, 1995: Fairy tale or reality ? Medtronic Chronobiology Seminar #8, April 1995, 12 pp. text, 18 figures. (http://www.msi.umn.edu/~halberg/)
43. Macey SL. (ed.). Encyclopedia of Time. Garland Publishing, New York, 1994, 699 pp.
44. Cornélissen G, Halberg F. Chronomedicine. In: Encyclopedia of Biostatistics, Armitage P, Colton T (editors-in-chief), v. 1, John Wiley & Sons Ltd., Chichester, UK, 1998, pp. 642-649.
45. Halberg F, Cornélissen G. Chronobiology, rhythms, clocks, chaos, aging, and other trends. In: Maddox G. (ed.) Encyclopedia of Aging, 3rd ed. Springer, New York, 2001, pp. 196-201.
46. Cornélissen G, Halberg F. Broadly pertinent chronobiology methods quantify phosphate dynamics (chronome) in blood and urine. Clin Chem 38: 329-333, 1992.
47. Halberg F, Cornélissen G, Carandente F. Chronobiology leads toward preventive health care for all: cost reduction with quality improvement. A challenge to education and technology *via* chronobiology. Chronobiologia 18: 187-193, 1991.
48. Cornélissen G, Halberg F, Schwartzkopff O, et al. Chronomes, time structures, for chronobioengineering for "a full life". Biomedical Instrumentation & Technology 33: 152-187, 1999.
49. Halberg F, Cornélissen G, Otsuka K, et al., International BIOCOS Study Group. Cross-spectrally coherent ~10.5- and 21-year biological and physical cycles, magnetic storms and myocardial infarctions. Invited presentation, NATO Advanced Study Institute on Space Storms and Space Weather Hazards, Crete, Greece, June 19-29, 2000. Neuroendocrinol Lett 21: 233-258, 2000.

50. Halberg F, Gupta BD, Haus E, et al. Steps toward a cancer chronopolytherapy. In: Proc. XIV International Congress of Therapeutics, Montpellier, France, L'Expansion Scientifique Française, 1977, pp. 151-196.
51. Levi F, Halberg F, Nesbit M, Haus E, Levine H. Chrono-oncology. In: Neoplasms-Comparative Pathology of Growth in Animals, Plants and Man, H. Kaiser, ed., Williams and Wilkins, Baltimore, 1981, pp. 267-316.
52. Halberg F, Cornélissen G, Sothern RB, et al. International geographic studies of oncological interest on chronobiological variables. In: Neoplasms-Comparative Pathology of Growth in Animals, Plants and Man, H. Kaiser, ed., Williams and Wilkins, Baltimore, 1981, pp. 553-596.
53. Halberg Francine, Halberg J, Halberg E, Halberg Franz: Chronobiology, radiobiology and steps toward the timing of cancer radiotherapy. In: Cancer Growth and Progression, vol. 9, ch. 19, H. Kaiser series ed., A.L. Goldson volume ed., Kluwer Academic Publ., Dordrecht, 1989, pp. 227-253.
54. Cornélissen G, Halberg F. The chronobiologic pilot study with special reference to cancer research: Is chronobiology or, rather, its neglect wasteful? In: Cancer Growth and Progression, vol. 9, ch. 9, H. Kaiser series ed., A.L. Goldson volume ed., Kluwer Academic Publ., Dordrecht, 1989, pp. 103-133.
55. Bartter FC, Delea CS, Baker W, Halberg F, Lee JK. Chronobiology in the diagnosis and treatment of mesor-hypertension. Chronobiologia 3: 199-213, 1976.
56. Levine H, Halberg F. Circadian rhythms of the circulatory system. Literature review. Computerized case study of transmeridian flight and medication effects on a mildly hypertensive subject. U.S. Air Force Report SAM-TR-72-3, April 1972, 64 pp.
57. 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 51: 329-335, 1952.
58. Halberg F, Nelson W, Cornélissen G, Haus E, Scheving LE, Good RA. On methods for testing and achieving cancer chronotherapy. Cancer Treatment Rep. 63: 1428-1430, 1979.
59. Halberg F. Biological as well as physical parameters relate to radiology. Guest Lecture, Proc. 30th Ann. Cong. Rad., January 1977, Post-Graduate Institute of Medical Education and Research, Chandigarh, India, 8 pp.
60. Halberg E, Halberg J, Halberg Francine, Sothern RB, Levine H, Halberg F. Familial and individualized longitudinal autorhythmometry for 5 to 12 years and human age effects. J Gerontol 36: 31-33, 1981.
61. Halberg E, Halberg Francine, Halberg J, Halberg F. Forging chronobiology and pediatrics as well as geriatrics: a birthday greeting for Theodor Hellbrügge. Int J Chronobiol 6: 135-143, 1979.
62. In vivo, v. 7, 1993. Frontispiece. Pp. 269-416.
63. Elg S, Halberg E, Ramakrishnan R, et al. Marker rhythmometry with macrophage colony stimulating factor (M-CSF). Chronobiologia 18: 141-152, 1991.
64. Cornélissen G, Halberg E, Long HJ III, et al. Toward a chronotherapy of ovarian cancer with taxol. Part I: Basic background. Chronobiologia 18: 153-166, 1991.
65. Halberg E, Long HJ III, Cornélissen G, et al. Toward a chronotherapy of ovarian cancer with taxol: Part II: Test pilot study on CA125. Chronobiologia 19: 17-42, 1992.
66. Cornélissen G, Halberg F, Halberg E, et al. Toward a chronotherapy of ovarian cancer: Part III: Salivary CA125 for chronochemotherapy by efficacy. Chronobiologia 19: 131-149, 1992.

67. Halberg E, Cornélissen G, Haus E, et al. Amplification [on comments by Berry DA. Power of chronobiologic pilots: a statistician's opinion. Chronobiologia 20: 213-214, 1993]. Chronobiologia 20: 214-218, 1993.
68. Halberg E, Cornélissen G, Bakken E, Halberg F. Chrononeuroimmunomodulation (chrono-NIM): lead-lag cross-correlations of mental state and tumor burden. Chronobiologia 21: 144-145, 1994.
69. Kennedy BJ. A lady and chronobiology. Chronobiologia 20: 139-144, 1993.
70. Cornélissen G, Halberg E, Klee GG, et al. Changes in ovarian tumor markers during 24-h drug infusion assess times of treatment efficacy. Abstract, XV Congreso Nacional de Quimica Clinica, Queretaro, Mexico, May 1-5, 1992. Bioquimia 17: 51-52, 1992.
71. Halberg F. The Montalbetti Lecture: When to treat: tumor marker chronomes, putative tools for treatment timing by efficacy. XV Congreso Nacional de Quimica Clinica, Queretaro, Mexico, May 1-5, 1992. Bioquimia 17: 36-42, 1992.
72. Halberg F, Cornélissen G, Bingham C, Fujii S, Halberg E. From experimental units to unique experiments: chronobiologic pilots complement large trials. in vivo 6: 403-428, 1992.
73. Bingham C, Cornélissen G, Halberg F. Power of "Phase 0" chronobiologic trials at different signal-to-noise ratios and sample sizes. Chronobiologia 20: 179-190, 1993.
74. Halberg F, Bingham C, Cornélissen G. Clinical trials: the larger the better? Chronobiologia 20: 193-212, 1993.
75. Hawkins DM. Self-starting CUSUM charts for location and scale. The Statistician 36: 299-315, 1987.
76. Hawkins DM. Cumulative SUM control charting: an underutilized SPC tool. Quality Engineering 5: 463-477, 1993.
77. Cornélissen G, Halberg F, Hawkins D, Otsuka K, Henke W. Individual assessment of antihypertensive response by self-starting cumulative sums. J Medical Engineering & Technology 21: 111-120, 1997.
78. Fine RL, Cornélissen G, Haus E, et al. Erna-test: chemosensitivity assays and marker rhythmometry target cancer treatment in time and kind. Abstract, 16th World Congress of Anatomic and Clinical Pathology, Acapulco, Mexico, October 5-9, 1993.
79. Von Hoff DD, Kronmal R, Salmon SE, et al. A Southwest Oncology Group study on the use of a human tumor cloning assay for predicting response to patients with ovarian cancer. Cancer 67: 20-27, 1991.
80. Levi F, Zidani R, Brienza S, et al. A multicenter evaluation of intensified, ambulatory, chronomodulated chemotherapy with oxaliplatin, 5-fluorouracil, and leucovorin as initial treatment of patients with metastatic colorectal carcinoma. International Organization for Cancer Chronotherapy. Cancer 85: 2532-2540, 1999.
81. Vollrath L. Biology of the pineal gland and melatonin in humans. In: The Pineal Gland and Cancer: Neuroimmunoendocrine Mechanisms in Malignancy (Bartsch C, Bartsch H, Blask DE, Cardinali DP, Hrushesky WJM, Mecke W, eds). Heidelberg: Springer, 2000, pp. 3-49.
82. Halberg F, Cornélissen G, Halberg E, Halberg J, Delmore P, Shinoda M, Bakken E. Chronobiology of human blood pressure. Medtronic Continuing Medical Education Seminars, 1988, 4th ed., 242 pp.
83. Halberg F, Bakken E, Cornélissen G, et al. Chronobiologic blood pressure assessment with a cardiovascular summary, the sphygmochron. In: Blood Pressure Measurements (Meyer-Sabellek W, Anlauf M, Gotzen R, Steinfeld L, eds), Steinkopff Verlag, Darmstadt, FRG, 1990, pp. 297-326.

84. Cornélissen G, Otsuka K, Halberg F. Blood pressure and heart rate chronome mapping: a complement to the human genome initiative. In: Chronocardiology and Chronomedicine: Humans in Time and Cosmos (Otsuka K., Cornélissen G., Halberg F. eds). Life Science Publishing, Tokyo, 1993, pp. 16-48.
85. Haus E, Haus M Sr, Cornélissen G, Wu JY, Halberg F. A longitudinal view with 16,944 sets of self-measurements of the aging human circulation. In: Chronocardiology and Chronomedicine: Humans in Time and Cosmos (Otsuka K, Cornélissen G, Halberg F. eds), Life Science Publishing, Tokyo, 1993, pp. 99-102.
86. Raab F, Schaffer E, Cornélissen G, Halberg F. More on the dentist's role in the prevention of high blood pressure. Chronobiologia 20: 245-250, 1993.
87. Halberg F, Cornélissen G, Halpin C, et al. Fleeting "monitor ", "conflict " or "grief-associated" blood pressure disorders: MESOR-hypertension and circadian hyperamplitudetension (CHAT). EuroRehab 6: 225-240, 1996.
88. Halberg F, Cornélissen G, Haus E, et al. Clinical relevance of about-yearly changes in blood pressure and the environment. Int J Biometeorol 39: 161-175, 1996.
89. Portela A, Cornélissen G, Halberg F, Menéndez JC. Can blood pressure variability be reconciled with the interpretation of single measurements versus fixed thresholds? EuroRehab 6: 33-48, 1996.
90. Portela A, Northrup G, Halberg F, Cornélissen G, Wendt H, Melby JC, Haus E. Changes in human blood pressure with season, age and solar cycles: a 26-year record. Int J Biometeorol 39: 176-181, 1996.
91. Cornélissen G, Halberg F, Wall D, Siegelova J, Zaslavskaya RM. How long to screen: ice hockey game and transient circadian hyperamplitudetension, CHAT. Scripta medica (Brno) 70: 189-198, 1997.
92. Raab FJ, Schaffer EM, Guillaume-Cornélissen G, Halberg F. Interpreting vital sign profiles for maximizing patient safety during dental visits. JADA 129: 461-469, 1998.
93. Halberg F, Cornélissen G, Wrbsky P, et al. About 3.5-day (circasemiseptan) and about 7-day (circaseptan) blood pressure features in human prematurity. Chronobiologia 21: 146-151, 1994.
94. Gubin D, Cornélissen G, Halberg F, Gubin G, Uezono K, Kawasaki T. The human blood pressure chronome: a biological gauge of aging. In vivo 11: 485-494, 1997.
95. Halberg F, Tamura K, Cornélissen G. Chronobioengineering toward a cost-effective quality health care. Frontiers Med Biol Eng 6: 83-102, 1994.
96. Halberg F, Cornélissen G, Sonkowsky RP, Lanzoni C, Galvagno A, Montalbini M, Schwartzkopff O. Chrononursing (chronutrics), psychiatry and language. New Trends in Experimental and Clinical Psychiatry 14: 15-26, 1998.
97. Halberg F, Cornélissen G, Otsuka K, et al. Rewards in practice from recycling heart rate, ectopy, ischemia, and blood pressure information. J Medical Engineering & Technology 21: 174-184, 1997.
98. Otsuka K, Cornélissen G, Halberg F, Oehlert G. Excessive circadian amplitude of blood pressure increases risk of ischemic stroke and nephropathy. J Medical Engineering & Technology 21: 23-30, 1997.
99. Chen CH, Cornelissen G, Halberg F, Fiser B. Left ventricular mass index as "outcome" related to circadian blood pressure characteristics. Scripta medica (Brno) 71: 183-189, 1998.
100. Chen CH, Cornélissen G, Siegelova J, Halberg F. Does overswinging provide an early warning of cardiovascular disease risk when non-dipping may fail? A meta-analysis of 2039 cases. Abstract 11, MEFA, Brno, Czech Rep., Nov. 3-6, 1999.

101. Cornélissen G, Chen CH, Siegelova J, Halberg F. Vascular disease risk syndromes affecting both MESOR-normotensives and MESOR-hypertensives: a meta-analysis of 2039 cases. Abstract 12, MEFA, Brno, Czech Rep., Nov. 3-6, 1999.
102. Otsuka K, Cornélissen G, Halberg F. Circadian rhythmic fractal scaling of heart rate variability in health and coronary artery disease. Clinical Cardiology 20: 631-638, 1997.
103. Cornélissen G, Otsuka K, Chen C-H, et al. Nonlinear relation of the circadian blood pressure amplitude to cardiovascular disease risk. Scripta medica (Brno) 73: 85-94, 2000.
104. Cornélissen G, Otsuka K, Bakken EE, Halberg F, Siegelova J, Fiser B. CHAT and CSDD-HR (circadian standard deviation deficit): independent, synergistic vascular disease risks. MEFA 8th International Fair of Medical Technology and Pharmacy, Brno, Czech Republic, 7-10 Nov 2000, abstract 7.
105. Halberg F, Cornélissen G, Halberg J, et al. Circadian Hyper-Amplitude-Tension, CHAT: a disease risk syndrome of anti-aging medicine. J Anti-Aging Med 1: 239-259, 1998. (Editor's Note by Fossel M., p. 239.)
106. Cornélissen G., Halberg F. Impeachment of casual blood pressure measurements and the fixed limits for their interpretation and chronobiologic recommendations. Time-dependent Structure and Control of Arterial Blood Pressure. Ann NY Acad Sci 783: 24-46, 1996.
107. Bartter FC. Periodicity and medicine. In: Chronobiology (Scheving LE, Halberg F, Pauly JE eds). Igaku Shoin Ltd., Tokyo, 1974, pp. 6-13.
108. Watanabe Y, Cornélissen G, Halberg F, Otsuka K, Kikuchi T. Long-acting carteolol lowers circadian and circaseptan blood pressure (BP) amplitude (A) as well as MESOR. Abstract, X National Symposium, Indian Society for Chronobiology, B.J. Medical College, Pune, India, August 21-22, 1995, pp. 14-15.
109. Watanabe Y, Cornélissen G, Halberg F, Saito Yoshiaki, Fukuda K, Otsuka K, Kikuchi T. Chronobiometric assessment of autogenic training effects upon blood pressure and heart rate. Perceptual and Motor Skills 83: 1395-1410, 1996.
110. Watanabe Y, Cornélissen G, Halberg F, et al. Method and need for continued assessment of autogenic training effect upon blood pressure: case report. New Trends in Experimental and Clinical Psychiatry 12: 45-50, 1996.
111. Watanabe Y, Halberg F, Cornélissen G, et al. Self-hypnosis lowers blood pressure swinging and overswinging in circadian hyperamplitudetension (CHAT). EuroRehab 2: 83-94, 1996.
112. Halberg F, Smith HN, Cornélissen G, Delmore P, Schwartzkopff O, International BIOCOS Group. Hurdles to asepsis, universal literacy, and chronobiology-all to be overcome. Neuroendocrinol Lett 21: 145-160, 2000.
113. Halberg F, Cornélissen G, Schwartzkopff O, et al. Spin-offs from blood pressure and heart rate studies for health care and space research (review). in vivo 13: 67-76, 1999.
114. Otsuka K (organizer). Proceedings, 1st International Symposium, Workshop on Chronoastrobiology & Chronotherapy (Satellite Symposium, 7th Annual Meeting, Japanese Society for Chronobiology), Kudan, Chiyodaku, Tokyo, 11 Nov 2000, 138 pp.
115. Halberg F. The week in phylogeny and ontogeny: opportunities for oncology. In vivo 9: 269-278, 1995.
116. Halberg F, Breus TK, Cornélissen G, et al. Chronobiology in space. Keynote, 37th Ann. Mtg. Japan Soc. for Aerospace and Environmental Medicine, Nagoya, Japan, November 8-9, 1991. University of Minnesota/Medtronic Chronobiology Seminar Series, #1, December 1991, 21 pp. of text, 70 figures.

117. Roederer JG. Are magnetic storms hazardous to your health? Eos, Transactions, American Geophysical Union 76: 441, 444-445, 1995.
118. Vladimirskii BM, Narmanskii VYa, Temuriantz NA. Global rhythmics of the solar system in the terrestrial habitat. Biophysics 40: 731-736, 1995.
119. Ulmer W, Cornélissen G, Halberg F. Physical chemistry and the biologic week in the perspective of chrono-oncology. In vivo 9: 363-374, 1995.
120. Ulmer W. On the role of the interactions of ions with external magnetic fields in physiologic processes and their importance in chronobiology. In vivo 16: 31-36, 2002.
121. Díez-Noguera A, Cambras T, Cornélissen G, Halberg F. A biological week in the activity chronome of the weanling rat: a chrono-meta-analysis. Keynote, 4° Convegno Nazionale, Societa Italiana di Cronobiologia, Gubbio (Perugia), Italy, June 1-2, 1996, pp. 81-82.
122. Thaela M-J, Jensen MS, Cornélissen G, Halberg F, Nöddegaard F, Jakobsen K, Pierzynowski SG. Circadian and ultradian variation in pancreatic secretion of meal-fed pigs after weaning. J Animal Science 76(4): 1131-1139, 1998.
123. Fanjul Moles ML, Cornélissen G, Miranda Anaya M, Prieto Sagredo J, Halberg F. Larger infradian vs. circadian prominence of locomotor activity in young vs. older crayfish. Abstract, 6° Convegno Nazionale de Cronobiologia, Chianciano, Italy, November 27-28, 1998, p. 65.
124. Halberg F, Cornélissen G, Otsuka K, Schwartzkopff O. Physiological monitoring from bacteria and eukaryotic unicells to humans for chronoastrobiology and chronomedicine. Proceedings, 1st International Symposium, Workshop on Chronoastrobiology & Chronotherapy (Satellite Symposium, 7th Annual Meeting, Japanese Society for Chronobiology), Kudan, Chiyodakù, Tokyo, 11 Nov 2000, pp. 56-74.
125. DeVecchi A, Halberg F, Sothern RB, Cantaluppi A, Ponticelli C. Circaseptan rhythmic aspects of rejection in treated patients with kidney transplant. In: Chronopharmacology and Chronotherapeutics (Walker CA, Winget CM, Soliman KFA, eds). Florida A & M University Foundation, Tallahassee, Florida, 1981, pp. 339-353.
126. Hübner K. Kompensatorische Hypertrophie, Wachstum und Regeneration der Rattenniere. Ergebn allg Path path Anat 100: 1-80, 1967.
127. Levi F, Halberg F. Circaseptan (about 7-day) bioperiodicity -- spontaneous and reactive -- and the search for pacemakers. La Ricerca Clin Lab 12: 323-370, 1982.
128. Halberg F, Cornélissen G, Otsuka K, Gubin D, Zaslavskaya R, Johnson D. Basic and applied chronobiologic frontiers in 1996. Lettura magistrale, 4° Convegno Nazionale, Societa Italiana di Cronobiologia, Gubbio (Perugia), Italy, June 1-2, 1996, pp. 12-18.
129. Halberg F, Cornélissen G, Katinas GS, et al. Feedsidewards: intermodulation (strictly) among time structures, chronomes, in and around us, and cosmo-vasculo-neuroimmunity. About ten-yearly changes: what Galileo missed and Schwabe found. In: Conti A, Maestroni GJM, McCann SM, Sternberg EM, Lipton JM, Smith CC (eds.), Neuroimmunomodulation (Proc. 4th Int. Cong. International Society for Neuroimmunomodulation, Lugano, Switzerland, September 29-October 2, 1999). Ann NY Acad Sci 917: 348-376, 2000.
130. Cornélissen G, Broda H, Halberg F. Does *Gonyaulax polyedra* measure a week? Cell Biophysics 8: 69-85, 1986.
131. Cornélissen G, Halberg F, Edmunds L. Multiseptan modulations of circadian cell division and cell settling patterns in *Euglena*. Abstract, 2nd Int. Symp. of Chronobiology and Chronomedicine, Shenyang, China, Sept. 28-Oct. 2, 1996, pp. 63-64.

132. Woolum JC, Cornélissen G, Halberg F. Chronometaanalysis: enucleation changes the infradian-circadian amplitude ratio of *Acetabularia*. Abstract, 6° Convegno Nazionale de Cronobiologia, Chianciano, Italy, November 27-28, 1998, p. 64.
133. Faraone P, Starbuck S, Katinas GS, et al. Astrophysical influences on sectoring in colonies of microorganisms. Scripta medica (Brno) 74: 107-114, 2001.
134. Syutkina E.V., Cornélissen G., Grigoriev A.E., et al. Neonatal intensive care may consider associations of cardiovascular rhythms with local magnetic disturbance. Scripta medica (Brno) 70: 217-226, 1997.
135. Cornélissen G, Halberg F, Wendt HW, et al. Resonance of about-weekly human heart rate rhythm with solar activity change. Biologia (Bratislava) 51: 749-756, 1996.
136. Cornélissen G, Breus TK, Bingham C, et al. Beyond circadian chronorisk: worldwide circaseptan-circasemiseptan patterns of myocardial infarctions, other vascular events, and emergencies. Chronobiologia 20: 87-115, 1993.
137. Viduetsky A, Cornélissen G, Bryskin Y, Gunas I, Halberg F. Gender and age differences with similar weekly pattern of myocardial infarction. Reports of Morphology 1: 91-92, 1999.
138. Dubrov AP. The geomagnetic field and life: geomagnetobiology. (Translated by F.L. Sinclair; translation edited by F.A. Brown Jr.) Plenum Press, New York, 1978, 318 pp.
139. Gamburtsev AG, Alexandrov SI, Belyakov AS, et al. Atlas of Natural Processes: Order and chaos in lithosphere and other spheres. Rossiiskaya akademia nauk, Moscow, 1994, 176 pp.
140. Stoupel E. Forecasting in Cardiology. John Wiley & Sons, New York, 1976, 141 pp.
141. Mendoza B, Diaz-Sandoval R. Effects of solar activity on myocardial infarction deaths in low geomagnetic latitude regions. Natural Hazards 32: 25-36, 2004.
142. Cornélissen G, Halberg F, Gheonjian L, et al. Schwabe's ~10.5- and Hale's ~21-year cycles in human pathology and physiology. In: Long- and Short-Term Variability in Sun's History and Global Change (Schröder W, ed). Bremen: Science Edition, 2000: 79-88.
143. Baevsky RM, Petrov VM, Cornélissen G, et al. Meta-analyzed heart rate variability, exposure to geomagnetic storms, and the risk of ischemic heart disease. Scripta medica (Brno) 70: 199-204, 1997.
144. Otsuka K, Cornélissen G, Zhao ZY, et al. Rhythm and trend elements in the time structure, chronome, of heart rate variability. Geronto-Geriatrics 2: 31-48, 1999.
145. Kleiger RE, Miller JP, Bigger JT Jr, Moss AJ, Multicenter Post-Infarction Research Group: Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. Am J Cardiol 59, 256-262, 1987.
146. Cornélissen G, Bakken E, Delmore P, et al. From various kinds of heart rate variability to chronocardiology. Am J Cardiol 66: 863-868, 1990.
147. Algra A, Tijssen JG, Roelandt JR, Pool J, Lubsen J. Heart rate variability from 24-hour electrocardiography and the 2-year risk for sudden death. Circulation 88, 180-185, 1993.
148. Tsuji H., Venditti FJ, Manders ES, Evans JC, Larson MG, Feldman CL, Levy D. Reduced heart rate variability and mortality risk in an elderly cohort. The Framingham Heart Study. Circulation 90: 878-883, 1994.
149. Delyukov A, Gorgo Yu, Cornélissen G, Otsuka K, Halberg F. Natural environmental associations in a 50-day human electrocardiogram. Int J Biometeorol, 45: 90-99, 2001.

150. Otsuka K, Cornélissen G, Weydahl A, et al. Geomagnetic disturbance associated with decrease in heart rate variability in subarctic area. Biomed & Pharmacother 55 (Suppl 1): 51s-56s, 2001.
151. Demaine C, Semm P. Magnetic fields abolish nychthemeral rhythmicity of responses of Purkinje cells to the pineal hormone melatonin in the pigeon's cerebellum. Neuroscience Letters 72: 158-162, 1986.
152. Reiter RJ. Reported biological consequences related to the suppression of melatonin by electric and magnetic field exposure. Integrative Physiological & Behavioral Science 30: 314-330, 1995.
153. Scaiano JC. Exploratory laser flash photolysis study of free radical reactions and magnetic field effects in melatonin chemistry. J. Pineal Res. 19: 189-195, 1995.
154. Graham C, Cook MR, Riffle DW. Human melatonin during continuous magnetic field exposure. Bioelectromagnetics 18: 166-171, 1997.
155. Rapoport SI, Malinovskaia NK, Oraevsky VN, Komarov FI, Nosovskii AM, Wetterberg L. [Effects of disturbances of natural magnetic field of the earth on melatonin production in patients with coronary heart disease.] Klinicheskaia Meditsina 75: 24-26, 1997.
156. Burch JB, Reif JB, Yost MG, Keefe TJ, Pitrat CA. Reduced excretion of a melatonin metabolite in workers exposed to 60 Hz magnetic fields. Am. J. Epidemiol. 150: 27-36, 1999.
157. Reuss S, Semm P, Vollrath L. Different types of magnetically sensitive cells in the rat pineal gland. Neurosci. Lett. 40: 23-26, 1983.
158. Semm P. Neurobiological investigations on the magnetic sensitivity of the pineal gland in rodents and pigeons. Comp. Biochem. Physiol. 76: 683-689, 1983.
159. Welker HA, Semm P, Willig RP, Commentz JC, Witschko W, Vollrath L. Effects of an artificial magnetic field on serotonin N-acetyltransferase activity and melatonin content of the rat pineal gland. Exp. Brain Res. 50: 426-432, 1983.
160. Olcese J, Reuss S, Vollrath L. Evidence for the involvement of the visual system in mediating magnetic field effects on pineal melatonin synthesis in the rat. Brain Res. 333: 382-384, 1985.
161. Sakotnik A, Liebmann PM, Stoschitzky K, Lercher P, Schauenstein K, Klein W, Eber B. Decreased melatonin synthesis in patients with coronary artery disease. Eur. Heart J. 20: 1314-1317, 1999.
162. Wetterberg L, Halberg F, Halberg E, et al. Circadian characteristics of urinary melatonin from clinically healthy women at different civilization disease risk. Acta med scand 220: 71-81, 1986.
163. Tarquini B, Cornélissen G, Perfetto F, Tarquini R, Halberg F. Chronome assessment of circulating melatonin in humans. In vivo 11: 473-484, 1997.
164. Grafe A. Einige charakterische Besonderheiten des geomagnetischen Sonneneruptionseffektes. Geofisica Pura e Applicata 40: 172-179, 1958.
165. Russell CT, McPherron RL. Semiannual variation of geomagnetic activity. J. Geophys. Res. 78: 92-108, 1973.
166. Martikainen H, Tapanainen J, Vakkuri O, Leppäluoto J, Huhtaniemi I. Circannual concentrations of melatonin, gonadotrophins, prolactin and gonadal steroids in males in a geographical area with a large annual variation in daylight. Acta endocrinol. (Copenhagen) 109: 446-450, 1985.
167. Randall W. The solar wind and human birth rate: a possible relationship due to magnetic disturbances. Int. J. Biometeorol. 34: 42-48, 1990.

168. Bergiannaki J, Paparrigopoulos TJ, Stefanis CN. Seasonal pattern of melatonin excretion in humans: relationship to daylength variation rate and geomagnetic field fluctuations. Experientia 52: 253-258, 1996.
169. Bartsch H, Bartsch C, Mecke D, Lippert TH. Seasonality of pineal melatonin production in the rat: possible synchronization by the geomagnetic field. Chronobiology int. 11: 21-26, 1994.
170. Wetterberg L, Bratlid T, Knorring Lv, Eberhard G, Yuwiler A. A multinational study of the relationships between nighttime urinary melatonin production, age, gender, body size, and latitude. Eur. Arch. Psychiatr. Neurosci. 249: 256-262, 1999.
171. King DP, Takahashi JS. Molecular genetics of circadian rhythms in mammals. Ann Rev Neuroscience 23: 713-742, 2000.
172. Schwartz WJ, Carpino A Jr, de la Iglesia HO, Baler R, Klein DC, Nakabeppu Y, Aronin N. Differential regulation of fos family genes in the ventrolateral and dorsomedial subdivisions of the rat suprachiasmatic nucleus. Neuroscience 98: 535-547, 2000.
173. Konopka RJ, Kyriacou CP, Hall JC. Mosaic analysis in the Drosophila CNS of circadian and courtship-song rhythms affected by a period clock mutation. J Neurogenetics 11: 117-139, 1996.
174. Kyriacou CP, Hall JC. Circadian rhythm mutations in *Drosophila melanogaster* affect a short-term fluctuation in the male's courtship song. Proc Nat Acad Sci USA 77: 6929-6933, 1980.
175. Park DH, Somers DE, Kim YS, et al. Control of circadian rhythms and photoperiodic flowering by the Arabidopsis GIGANTEA gene. Science 285: 1579-1582, 1999.
176. Majercak J, Sidote D, Hardin PE, Edery I. How a circadian clock adapts to seasonal decreases in temperature and day length. Neuron 24: 219-310, 1999.
177. Nelson DC, Lasswell J, Rogg LE, Cohen MA, Bartel B. FKF1, a clock-controlled gene that regulates the transition to flowering in *Arabidopsis*. Cell 101: 331-340, 2000.
178. Jeon M, Gardner HF, Miller EA, Deshler J, Rougvie AE. Similarity of the C. *elegans* developmental timing protein LIN-42 to circadian rhythm proteins. Science 286: 1141-1146, 1999.
179. Halberg F, Cornélissen G, Katinas G, et al. Transdisciplinary unifying implications of circadian findings in the 1950s. J Circadian Rhythms 1: 2. 61 pp., 2003. www.JCircadianRhythms.com/content/pdf/1740-3391/1/2.pdf
180. Halberg F, Cornélissen G, Otsuka K, Schwartzkopff O, Halberg J, Bakken EE. Chronomics. Biomedicine and Pharmacotherapy 55 (Suppl 1): 153-190, 2001.
181. Halberg F, Cornélissen G, Wang ZR, et al. Chronomics: circadian and circaseptan timing of radiotherapy, drugs, calories, perhaps nutriceuticals and beyond. J Exp Therapeutics Oncol 3: 223-260, 2003.
182. Halberg F, Cornélissen G, Wall D et al. Engineering and governmental challenge: 7-day/24-hour chronobiological blood pressure and heart rate screening. Biomedical Instrumentation & Technology 36: Part I, 89-122, Part II, 183-197, 2002.
183. Cornélissen G, Delmore P, Halberg F. Health Watch 3. University of Minnesota/Medtronic, Minneapolis, MN, 31pp., 2004.
184. Schaffer E, Cornélissen G, Rhodus N, Halhuber M, Watanabe Y, Halberg F. Outcomes of chronobiologically normotensive dental patients: a 7-year follow-up. JADA 132: 891-899, 2001.
185. Scarpelli PT, Romano S, Livi R, et al. Instrumentation for human blood pressure rhythm assessment by self- measurement - In: Chronobiotechnology and Chronobiological Engineering, Scheving LE, Halberg F, Ehret CF (eds)., Martinus Nijhoff, The Netherlands, pp. 304-309, 1987.

186. Müller-Bohn T, Cornélissen G, Halhuber M, et al. CHAT und Schlagfall. Deutsche Apotheker Zeitung 142: 366-370, 2002.
187. Watanabe Y, Cornélissen G, Watanabe M, et al. Effects of autogenic training and antihypertensive agents on circadian and circaseptan variation of blood pressure. Clin Exp Hypertens 25: 405-412, 2003.
188. Shinagawa M, Kubo Y, Otsuka K, Ohkawa S, Cornélissen G, Halberg F. Impact of circadian amplitude and chronotherapy: relevance to prevention and treatment of stroke. Biomedicine and Pharmacotherapy 55 (Suppl 1): 125-132, 2001.
189. Halberg F, Cornélissen G, Otsuka K et al., International BIOCOS Study Group. Cross-spectrally coherent ~10.5- and 21-year biological and physical cycles, magnetic storms and myocardial infarctions. Neuroendocrinol Lett 21: 233-258, 2000.
190. Cornélissen G, Halberg F, Breus T, et al. Non-photic solar associations of heart rate variability and myocardial infarction. J Atmos Solar-Terr Phys 64: 707-720, 2002.
191. Cornélissen G (ed.), Schwartzkopff O, Niemeyer-Hellbrügge P, Halberg F (co-editors). Time structures -- chronomes -- in child development. International Interdisciplinary Conference, Nov. 29-30, 2002, Munich, Germany. Neuroendocrinol Lett 24 (Suppl 1). 256 pp., 2003.
192. Halberg F, Cornélissen G, Bingham C, et al. Chronomics: Imaging in time by phase synchronization reveals wide spectral-biospheric resonances beyond short rhythms. ("Wenn man über kurze Rhythmen hinausgeht") In memoriam - lost future: Dr.-Ing. habil. Dr. rer. nat. Barbara Schack: 1952-2003. Neuroendocrinol Lett 24: 355-380, 2003.
193. Halberg F, Cornélissen G, Schack B. Self-experimentation on chronomes, time structures; chronomics for health surveillance and science: also transdisciplinary civic duty? Behavioral and Brain Sciences, http://www.bbsonline.org/Preprints/Roberts/Commentators/Halberg.html
194. Halberg F, Cornélissen G, Schack B, et al. Blood pressure self-surveillance for health also reflects 1.3-year Richardson solar wind variation: spin-off from chronomics. Biomed Pharmacother 57 (Suppl 1): 58s-76s, 2003.
195. Cornélissen G, Masalov A, Halberg F, et al. Multiple resonances among time structures, chronomes, around and in us. Is an about 1.3-year periodicity in solar wind built into the human cardiovascular chronome? Human Physiology 30: 86-92, 2004.
196. Cornélissen G, Engebretson M, Johnson D, Otsuka K, Burioka N, Posch J, Halberg F. The week, inherited in neonatal human twins, found also in geomagnetic pulsations in isolated Antarctica. Biomedicine and Pharmacotherapy 55 (Suppl 1): 32-50, 2001.
197. Cornélissen G, Hillman D, Katinas G, et al. Geomagnetics and society interact in weekly and broader multiseptans underlying health and environmental integrity. Biomed Pharmacother 56 (Suppl 2), 319s-326s, 2002.
198. Matsuto T. Weak coupling of a circadian clock gives rise to an infradian rhythm. Proc 4th International Symposium, Workshop on Chronoastrobiology and Chronotherapy, Kamo T (ed.) Dept of Neurology, Seibu Hospital, Yokohama, 8 Nov 2003, pp. 19-20.
199. Shinoda H, Ohtsuka-Isoya M, Cornélissen G, Halberg F. Chronome carved into murine dentin includes prominent about-weekly (circaseptan) component free-running in continuous light. Proc 3rd International Symposium, Workshop on Chronoastrobiology and Chronotherapy, Eriguchi M (ed.) Tokyo University, 9 Nov 2002, Abstract 13.
200. Shinoda H, Ohtsuka-Isoya M, Cornélissen G, Halberg F. Putative circaseptans or other infradians in murine dentin accretion and the suprachiasmatic nuclei. Neuroendocrinol Lett 24 (Suppl 1): 208-211, 2003.

TABLE 2A: RELATIVE RISK (RR) OF MORBIDITY ASSOCIATED WITH DBP-CHAT AND DHRV, SINGLY OR IN COMBINATION*

	Group		
	DBP-CHAT-only	DHRV-only	DBP-CHAT and DHRV
N of patients	20	19	5
All morbid events	4.43 2.13 - 9.19	5.33 2.71 - 10.46	10.12 5.51 - 18.58
Cerebral ischemic events	9.49 [2.28 - 39.49]	13.32 [3.61 - 49.11]	37.95** [11.35 - 126.9]
Coronary artery disease	3.16 [0.72 - 13.91]	8.32 [3.01 - 22.97]	6.32 [0.96 - 41.49]
Nephropathy	6.32 [2.08 - 19.20]	4.99 [1.44 - 17.30]	18.97** [7.06 - 50.99]
Retinopathy	2.11 [0.51 - 8.78]	1.11 [0.15 - 8.09]	4.22 [0.67 - 26.50]

*RR listed with 95% confidence interval;

DBP: diastolic blood pressure;

CHAT: circadian hyper-amplitude-tension;

DHRV: decreased heart rate variability.

Total number of patients: 297.

**RR associated with combined DBP-CHAT and DHRV is statistically significantly larger than RR associated with either DBP-CHAT or DHRV alone.

Data of K Otsuka

TABLE 2B: RELATIVE RISK (RR) AND 95% CONFIDENCE INTERVAL (CI) OF DIASTOLIC CIRCADIAN HYPER-AMPLITUDE-TENSION (D-CHAT), DECREASED HEART RATE VARIABILITY (DHRV), AND EXCESSIVE PULSE PRESSURE (EPP), ALONE OR IN COMBINATION*

Group 1: Reference (N_1) Risk?	Group 2: Test (N_2) Risk?	RR	[95% CI]
None (214)	D-CHAT (17)	6.294	[2.108; 18.794]
None (214)	DHRV (13)	8.231	[2.847; 23.797]
None (214)	EPP (39)	8.231	[3.600; 18.819]
None (214)	D-CHAT & DHRV (2)	13.375	[2.857; 62.621]
None (214)	D-CHAT & EPP (3)	26.750	[13.554; 52.795]
None (214)	DHRV & EPP (6)	17.833	[7.364; 43.189]
None (214)	D-CHAT & DHRV & EPP (3)	26.750	[13.554; 52.795]
D-CHAT (17)	D-CHAT & DHRV (2)	*2.125*	*[0.417; 10.840]*
D-CHAT (17)	D-CHAT & EPP (3)	4.250	[1.804; 10.013]
D-CHAT (17)	D-CHAT & DHRV & EPP (3)	4.250	[1.804; 10.013]
DHRV (13)	DHRV & D-CHAT (2)	*1.625*	*[0.325; 8.113]*
DHRV (13)	DHRV & EPP (6)	*2.167*	*[0.803; 5.846]*
DHRV (13)	D-CHAT & DHRV & EPP (3)	3.250	[1.438; 7.345]
EPP (39)	EPP & D-CHAT (3)	3.250	[2.030; 5.204]
EPP (39)	EPP & DHRV (6)	2.167	[1.038; 4.523]
EPP (39)	D-CHAT & DHRV & EPP (3)	3.250	[2.030; 5.204]

*Assessed in population of 297 patients, among whom 39 had a morbid event during the following 6 years. RR is computed as ratio of incidence of morbid event in Group 2 versus that of Group 1. A 95% CI not overlapping 1 indicates statistically significant increase in risk in Group 2 versus Group 1.

D-CHAT is defined as circadian amplitude of diastolic blood pressure (BP) above the upper 95% prediction limit of clinically healthy peers matched by gender and age;

DHRV is defined as 48-hour standard deviation of heart rate in the lowest 7^{th} percentile of distribution;

EPP is defined as a pulse pressure (MESOR of Systolic BP – MESOR of Diastolic BP) above 60 mm Hg, where the MESOR is a chronome-adjusted mean value.

Data of K Otsuka.

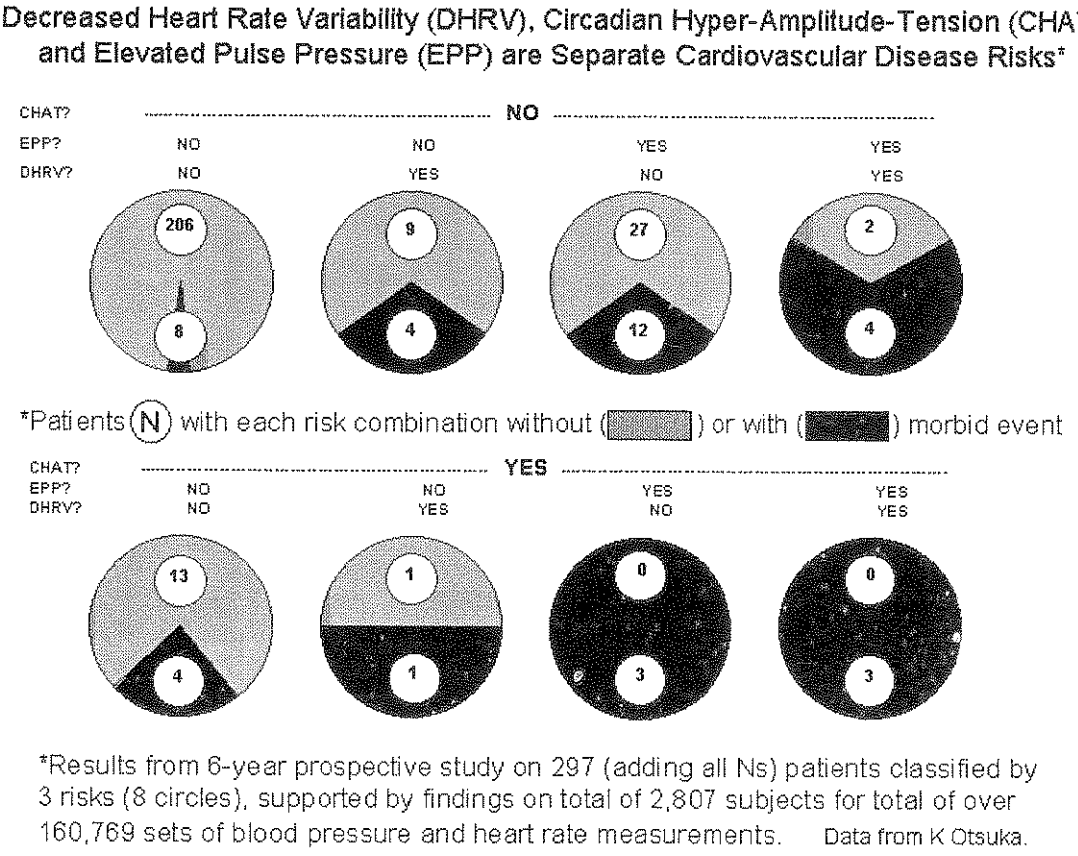


Figure 1: CHAT is one of several conditions related to the *variability* in BP and/or HR that is associated with an increase in vascular disease risk. Too large a pulse pressure (the difference between SBP and DBP, when the heart contracts or relaxes, or the extent of change in pressure during a cardiac cycle) and a decreased HR variability (defined as the standard deviation of HR) are two other such conditions. Vascular disease risk is elevated in the presence of any one of these three risk factors, and it is elevated further when more than a single risk factor is present, suggesting that these abnormalities in *variability* of BP and HR are mostly independent and additive. © *Halberg*.

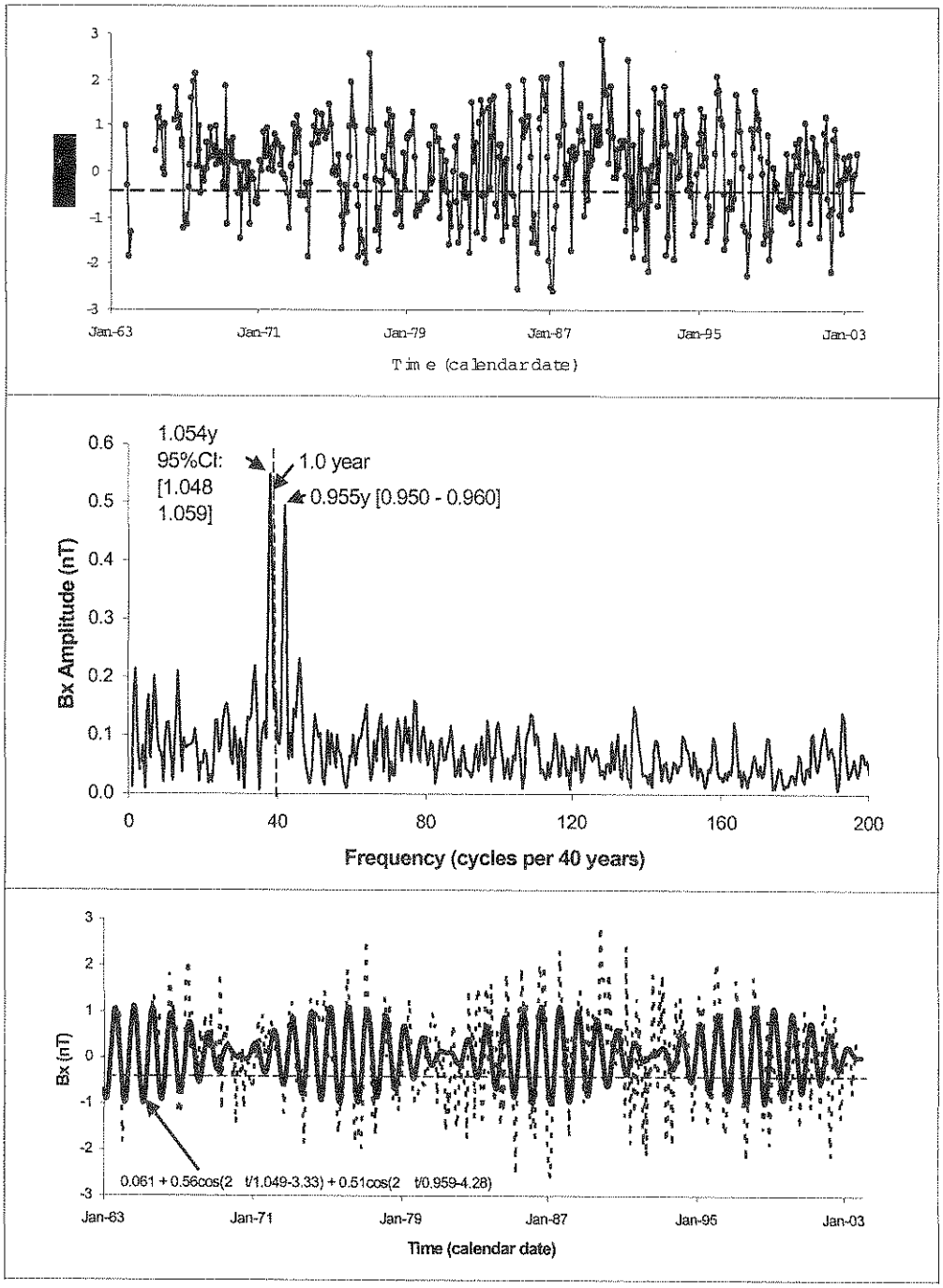


Figure 2: Nearly symmetrical nearcis- and neartransyear in the Interplanetary Magnetic Field (IMF). Daily values of Bx GSE, GSM, the magnitude of the horizontal component of the IMF, retrieved from OMNI 2, averaged monthly, from Jan 1963 to Dec 2003 (http://nssdc.gsfc.nasa.gov/omniweb/ow.html) (top). A neartransyear and a nearcisyear are major components of almost equal prominence in the least squares spectrum of B_x (middle). The corresponding model, shown with the data (bottom), accounts for about 29% of the overall variance ($P<0.001$). These two components are also detected in B_y GSE and B_y GSM and in σ(Na/Np) (RMS-SD of the Alpha/Proton ratio), but not jointly in the other 36 variables of OMNI 2. © *Halberg*.

Year[1]	Reference	Study	Comment (summary of results)
1980	Halberg J. et al. (1)	Several groups of up to 40 spontaneously hypertensive stroke-prone Okamoto rats had 4-hourly blood pressure (BP) measurements for 24 hours, repeated on the same animals at different ages.	Before developing a high BP, the circadian BP amplitude may be elevated under the load of heating and handling for manual measurements of BP. An experimental laboratory model for circadian hyper-amplitude-tension (CHAT) was thus found.
1986	Scarpelli et al. (2; 3-7)	Several hundred schoolchildren in Florence, Italy, carried out self-measurements (SM) around the clock for one or several days: Results were replicated independently by investigators across the USA, in Portugal and in China by SM, and in Baltimore, USA, with ambulatory BP monitoring (ABPM).	Children with a **positive family history** of high BP and related cardiovascular diseases had a **larger circadian amplitude** of BP as compared to children with a negative family history.
1986	Halberg et al. (8)	20 neonates were monitored around the clock at 30-min intervals during the first week of life.	The difference in circadian BP amplitude as a function of the presence or absence of a family history of high BP (FHHBP) was detectable during the first week of life.
1990	Halberg et al. (9)	BP profiles around the clock were carried out on 164 babies in Italy and in various other geographic locations (Germany, Minnesota, Japan, Russia, Spain).	**Circaseptan differences** with FHHBP were found, but **circadian** differences were qualified as a putative function of Schwabe sunspot cycle stage or number.
1990	Halberg et al. (9)	39 babies exposed *in utero* to betamimetic drugs vs. 113 control babies were each monitored around the clock at 30-min intervals for 48 hours during first week of life.	Exposure *in utero* to **betamimetic drugs** is associated with an **elevated circadian BP amplitude** during the first week post-partum.
1995	Syutkina et al. (10)	18 children exposed *in utero* to betamimetic drugs vs. 25 control children in Moscow, Russia, studied by ABPM for 48 hours with measurements at 15-min intervals.	The elevated circadian BP amplitude associated with exposure *in utero* to betamimetic drugs seen during the first week of life lasts into adolescence.

[1]New findings opening a line of research in **underlined bold**, with year flush with left margin. Year for complementary results indented

Year[1]	Reference	Study	Comment (summary of results)
1989 • 1991 • 1994 • 1997	Cornélissen et al. (11) Halberg et al. (12) Cornélissen, Halberg (13) Cornélissen et al. (14, 15)	221 pregnant women in Minnesota, clinically healthy at the outset, were studied by ABPM with hourly measurements around the clock for 2 days in each trimester of pregnancy, providing 336 profiles.	In addition to an 8 mm Hg difference in mean value between women who will or will not develop complications (gestational hypertension, preeclampsia) observed during the first trimester of pregnancy, the occurrence of complications is also associated with BP profiles characterized by an **elevated circadian BP amplitude**. In particular, one case (JK) of CHAT where warning was not heeded, was followed 8 weeks later by severe pre-eclampsia, premature delivery and 26 months of hospitalization of offspring at a cost of about $1 million.
1992	Kumagai et al. (16)	30 men and 26 women 16-81 years of age, studied around the clock by ABPM in Tokyo, Japan. Characteristics of BP profile related to left ventricular mass index (LVMI).	MESOR-hypertension[2] may be preceded by a transient circadian amplitude elevation (an elevated circadian BP amplitude in the absence of an elevated mean value is observed at intermediate LVMI values, while an elevated MESOR is found only for the largest LVMI group).
1996 • 1997	Otsuka et al. (17) Otsuka et al. (18)	297 patients (121 normotensive + 176 treated hypertensive) studied around the clock by ABPM, with measurements at 15-min intervals for 48 hours, followed prospectively for 6 years. Characteristics of BP profile related to the actual incidence of adverse vascular events.	CHAT carries a relative risk of 8.2 (720% increase in risk) for ischemic cerebral events. This risk is larger than that associated with any other known risk factor, including MESOR-hypertension, and applies to normotensive as well as hypertensive patients. It is demonstrable in subpopulations of patients not presenting with any one of the other known risk factors.
1996	Watanabe et al. (19-21)	10 patients in Tokyo, Japan, monitored around the clock by ABPM with measurements at 15-min intervals for at least 1 week at monthly intervals while practicing autogenic training.	Autogenic training decreases an excessive circadian BP amplitude.
1991 • 1995	Tamura et al. (22) see also Halberg, Cornélissen (23)	81 hypertensive patients studied by ABPM before and on treatment with one of 8 anti-hypertensive drugs.	Some but not all anti-hypertensive drugs lower on excessive circadian BP amplitude.

[2]MESOR = midline-estimating statistic of rhythm, a time structure (chronome)-adjusted average.

Year[1]	Reference	Study	Comment (summary of results)
1996 • 1998	Cornélissen et al. (24) Halberg et al. (25)	Case report of fulminant CHAT in Minneapolis, MN, documented by ABPM.	CHAT had better predictive value than stress test and preceded by about 4 months the occurrence of a myocardial infarction in a 35-year-old man.
1997	Watanabe et al. (26)	392 patients in Tokyo, Japan, studied around the clock by ABPM with measurements at 15-minute intervals for 1-7 days.	CHAT is more likely to occur in patients with borderline hypertension (transition between normotension and hypertension, in keeping with results by Kumagai et al. (16)
1998	Chen et al. (27)	424 patients studied by ABPM in Taiwan; characteristics of BP profile related to LVMI.	**CHAT** is associated with an **elevated LVMI.**
1999	Cornélissen et al. (28); see also Cornélissen et al. (29); Halberg et al. (25)	Meta-analysis of data from 721 patients of Otsuka et al. (17, 18) and Chen et al. (27).	Whereas vascular disease risk is linearly related with the BP MESOR (rhythm-adjusted mean value), the relation with the circadian BP amplitude is nonlinear.
2001	Chen et al. (30); Cornélissen et al. (31)	2,039 patients (of whom 1,179 were untreated) studied by ABPM in Taiwan; characteristics of BP profile related to LVMI.	Confirmation of an elevation in LVMI in association with CHAT, and of nonlinear relation of LVMI with circadian BP amplitude.
2001	Schaffer et al. (32)	7-year follow-up (in terms of outcome) of 12 out of 24 dental patients who had been studied by ABPM with measurements at 15-minute intervals in 3 consecutive sessions of 4, 2 and 3 days for a total of 9 days bracketting 3 dental appointments	CHAT is associated with the occurrence of morbid events **(Only those who had abnormality in all 3 sessions had an adverse event.)**
1982	Orth-Gomér et al. (33)	50 patients with coronary artery disease (CAD) and 50 healthy controls undergoing 24-hour Holter monitoring in Stockholm, Sweden.	The circadian pattern of premature ventricular contractions differs between the two groups.
1990	Cornélissen et al. (34)	Meta-analysis of data from Huikuri et al. (35).	HRV is circadian periodic.
1991 • 1994	Halberg et al. (36); Cornélissen et al. (37)	Daily incidence of myocardial infarctions (MI) in Moscow, Russia, between 1 Jan 1979 and 31 Dec 1981 (N=85,819).	There is a **7% increase** in the **daily incidence of MI** after a **magnetic storm.**

Year[1]	Reference	Study	Comment (summary of results)
1996	Cornélissen et al. (38)	Healthy subjects	Remove-and-replace (subtraction and addition) approach results in damping and amplification of circaseptans in heart rate in keeping with circaseptans in solar wind.
1997	Otsuka et al. (39); see also Otsuka et al. (18)	10 patients with CAD and 11 healthy men studied by 24-hour Holter monitoring in Tokyo, Japan.	HRV, gauged by correlation dimension is reduced in patients with CAD; a decrease in HRV is demonstrable by night but not by day. In 297 patients studied by ABPM, a 550% increase in the risk of CAD was associated with a reduced HRV (24-h SD in lowest 8th percentile of distribution).
1997	Baevsky et al. (40)	Holter monitoring of 49 cosmonauts in space.	HRV is reduced during a magnetic storm.
1999	Cornélissen et al. (28)	129,205 deaths from MI in Minnesota from 1968 to 1996.	Excess of 220 deaths from MI per year during solar maxima vs. solar minima.
2000	Otsuka et al. (41)	Longitudinal electrocardiographic (ECG) record for 7 days of clinically healthy man	HRV is reduced during a magnetic storm as compared to quiet conditions; HRV decrease observed in [0.004-0.15] Hz (~46.5 s) but not in [0.15-0.40] Hz (~3.6 s) spectral region, suggesting involvement of the sympathetic rather than the parasympathetic nervous system
2000	Halberg et al. (42)	Reciprocal spectra in and around us	Circadecadals in many variables including SBP, DBP & HR
2001	Cornélissen et al. (31)	Meta-analysis of data from Otsuka et al. (12, 13)	CHAT and DHRV (reduced HRV) are independent disease risks
2003	Halberg et al. (43); Cornélissen et al. (44)	Meta-analysis of data from Otsuka et al. (12, 13)	Pulse pressure is yet another independent risk in combination with CHAT and DHRV

Conclusions:

1. The disease risk syndromes of circadian hyperamplitudetension (CHAT), of deficient heart rate variability and high pulse pressure can be compared with the O-rings of the space shuttle Challenger as warnings before disaster (28).

2. Timely detection and treatment of CHAT, DHRVs and high pulse pressure may reduce health care costs.

3. Magnetic storms contribute to myocardial infarctions.

4. A space weather report is indicated to prompt preventive measures.

1. Halberg J, Halberg E, Halberg F, Halberg Francine, Levine H, Haus E, Carandente F, Bartter FC, Delea C. Human and murine (SHRSP) blood pressure and telemetered core temperature variation and genetic disposition to mesor-hypertension. Chronobiologia 1978; 5: 342; Halberg J, Halberg E, Hayes DK, Smith RD, Halberg F, Delea CS, Danielson RS, Bartter FC. Schedule shifts, life quality and quantity modeled by murine blood pressure elevation and arthropod lifespan. Int J Chronobiol 1980; 7: 17-64.
2. Scarpelli PT, März W, Cornélissen G, Romano S, Livi R, Scarpelli L, Halberg E, Halberg F. Blood pressure self-measurement in schools for rhythmometric assessment of hyperbaric impact to gauge pressure "excess". In: Dal Palu C, Pessina AC (eds.), ISAM 1985, Proc. Int. Symp. Ambulatory Monitoring, Padua, March 29-30, 1985. Padua: CLEUP Editore, 1986; 229-237.
3. Halberg F, Ahlgren A, Haus E. Circadian systolic and diastolic hyperbaric indices of high school and college students. Chronobiologia 1984; 11: 299-309.
4. LaSalle D, Sothern RB, Halberg F. Sampling requirements for description of circadian blood pressure (BP) amplitude (A). Chronobiologia 1983; 10: 138.
5. Scheving LA, Scheving LE, Halberg F. Establishing reference standards by autorhythmometry in high school for subsequent evaluation of health status. In: Scheving LE, Halberg F, Pauly JE, editors. Chronobiology, Proc. Int. Soc. for the Study of Biological Rhythms, Little Rock, Ark. Stuttgart: Georg Thieme Publishers/Tokyo: Igaku Shoin Ltd., 1974; 386-393.
6. Johns KL, Halberg F, Cornélissen G, März W. Chronobiology at the American International School in Lisbon, Portugal. In: Halberg F, Reale L, Tarquini B, editors. Proc. 2nd Int. Conf. Medico-Social Aspects of Chronobiology, Florence, Oct. 2, 1984. Rome: Istituto Italiano di Medicina Sociale, 1986; 367-384.
7. Wan C, Wang Z, Cornélissen G, Halberg F. Age, gender and circadian or circasemidian blood pressure and heart rate variation of children. Chronobiologia 1992; 19: 121-129.
8. Halberg F, Cornélissen G, Bingham C, Tarquini B, Mainardi G, Cagnoni M, Panero C, Scarpelli P, Romano S, März W, Hellbrügge T, Shinoda M, Kawabata Y. Neonatal monitoring to assess risk for hypertension. Postgrad Med 1986; 79: 44-46.
9. Halberg F, Cornélissen G, Bakken E. Caregiving merged with chronobiologic outcome assessment, research and education in health maintenance organizations (HMOs). Progress in Clinical and Biological Research 1990; 341B: 491-549.
10. Syutkina EV, Cornélissen G, Halberg F, Grigoriev AE, Abramian AS, Yatsyk GV, Morozova NA, Ivanov AP, Shevchenko PV, Polyakov YA, Bunin AT, Sa?n SR, Maggioni C, Alvarez M, Fernandez O, Tarquini B, Mainardi G, Bingham C, Kopher R, Vernier R, Rigatuso J, Johnson D. Effects lasting into adolescence of exposure to betamimetics in utero. Clinical Drug Investigation 1995; 9: 354-362.
11. Cornélissen G, Kopher R, Brat P, Rigatuso J, Work B, Eggen D, Einzig S, Vernier R, Halberg F. Chronobiologic ambulatory cardiovascular monitoring during pregnancy in Group Health of Minnesota. Proc. 2nd Ann. IEEE Symp. on Computer-Based Medical Systems, Minneapolis, June 26-27, 1989. Washington DC: Computer Society Press, 1989; 226-237.
12. Halberg F, Cornélissen G, Carandente F. Chronobiology leads toward preventive health care for all: cost reduction with quality improvement. A challenge to education and technology via chronobiology. Chronobiologia 1991; 18: 187-193.

13. Cornélissen G, Halberg F. Introduction to Chronobiology. Medtronic Chronobiology Seminar #7, April 1994, 52 pp. (Library of Congress Catalog Card #94-060580; http://www.msi.umn.edu/~halberg/)
14. Cornélissen G, Halberg F, Bingham C, Kumagai Y. Toward engineering for blood pressure surveillance. Biomedical Instrumentation & Technology 1997; 31: 489-498.
15. Cornélissen G, Halberg F, Bingham C, Kumagai Y. More on software chronobioengineering for blood pressure surveillance. Biomedical Instrumentation & Technology 1997; 31: 511-513.
16. Kumagai Y, Shiga T, Sunaga K, Cornélissen G, Ebihara A, Halberg F. Usefulness of circadian amplitude of blood pressure in predicting hypertensive cardiac involvement. Chronobiologia 1992; 19: 43-58.
17. Otsuka K, Cornélissen G, Halberg F. Predictive value of blood pressure dipping and swinging with regard to vascular disease risk. Clinical Drug Investigation 1996; 11: 20-31.
18. Otsuka K, Cornélissen G, Halberg F, Oehlert G. Excessive circadian amplitude of blood pressure increases risk of ischemic stroke and nephropathy. J. Medical Engineering & Technology 1997; 21: 23-30.
19. Watanabe Y, Cornélissen G, Halberg F, Saito Yoshiaki, Fukuda K, Revilla M, Rodriguez C, Hawkins D, Otsuka K, Kikuchi T. Method and need for continued assessment of autogenic training effect upon blood pressure: case report. New Trends in Experimental and Clinical Psychiatry 1996; 12: 45-50.
20. Watanabe Y, Halberg F, Cornélissen G, Kikuchi T, Saito Y, Fukuda K, Revilla M Sr, Revilla M Jr, Rodriguez C, Wark DM, Otsuka K. Self-hypnosis lowers blood pressure swinging and overswinging in circadian hyperamplitudetension (CHAT). EuroRehab 1996; 2: 83-94.
21. Watanabe Y, Cornélissen G, Halberg F, Saito Yoshiaki, Fukuda K, Otsuka K, Kikuchi T. Chronobiometric assessment of autogenic training effects upon blood pressure and heart rate. Perceptual and Motor Skills 1996; 83: 1395-1410.
22. Tamura K, Kohno I, Saito Yuzo, Wakasugi K, Achiwa S, Imanishi Y, Cugini P, Halberg F. Antihypertensive individualized therapeutic strategy. Difesa Sociale 1991; 6: 109-124.
23. Halberg F, Cornélissen G, International Womb-to-Tomb Chronome Initiative Group: Resolution from a meeting of the International Society for Research on Civilization Diseases and the Environment (New SIRMCE Confederation), Brussels, Belgium, March 17-18, 1995: Fairy tale or reality ? Medtronic Chronobiology Seminar #8, April 1995, 12 pp. text, 18 figures. http://www.msi.umn.edu/~halberg/
24. Cornélissen G, Halberg F, Gubin D, Carandente F, Halberg J, Zaslavskaya R, Syutkin V, Kumagai Y, Watanabe Y, Otsuka K. Carpe diem mensuratem: Fulminant CHAT, its recognition a chronobiologic path to preventing a myocardial infarction? Abstract, 4° Convegno Nazionale, Societa Italiana di Cronobiologia, Gubbio (Perugia), Italy, June 1-2, 1996; 35-36.
25. Halberg F, Cornélissen G, Halberg J, Fink H, Chen C-H, Otsuka K, Watanabe Y, Kumagai Y, Syutkina EV, Kawasaki T, Uezono K, Zhao ZY, Schwartzkopff O. Circadian Hyper-Amplitude-Tension, CHAT: a disease risk syndrome of anti-aging medicine. J Anti-Aging Med 1998; 1: 239-259. (Editor's Note by Fossel M., p. 239.)
26. Watanabe Y, Cornélissen G, Halberg F, Bingham C, Siegelova J, Otsuka K, Kikuchi T. Incidence pattern and treatment of a clinical entity, overswinging or circadian hyperamplitudetension (CHAT). Scripta medica 1997; 70: 245-261.

27. Chen CH, Cornelissen G, Halberg F, Fiser B. Left ventricular mass index as "outcome" related to circadian blood pressure characteristics. Scripta medica 1998; 71: 183-189.
28. Cornélissen G, Halberg F, Schwartzkopff O, Delmore P, Katinas G, Hunter D, Tarquini B, Tarquini R, Perfetto F, Watanabe Y, Otsuka K. Chronomes, time structures, for chronobioengineering for "a full life". Biomedical Instrumentation & Technology 1999; 33: 152-187.
29. Cornélissen G, Otsuka K, Chen C-H, Kumagai Y, Watanabe Y, Halberg F, Siegelova J, Dusek J. Nonlinear relation of the circadian blood pressure amplitude to cardiovascular disease risk. Scripta medica (Brno) 2000; 73: 85-94.
30. Chen ChH, Cornélissen G, Siegelova J, Halberg F. Does overswinging provide an early warning of cardiovascular disease risk when non-dipping may fail ? A meta-analysis of 2039 cases. Scripta medica (Brno) 2001; 74: 75-80.
31. Cornélissen G, Chen ChH, Siegelova J, Halberg F. Vascular disease risk syndromes affecting both MESOR-normotensives and MESOR-hypertensives: a meta-analysis of 2039 cases. Scripta medica (Brno) 2001; 74: 81-86.
32. Schaffer E, Cornélissen G, Rhodus N, Halhuber M, Watanabe Y, Halberg F. Outcomes of chronobiologically normotensive dental patients: a 7-year follow-up. JADA 2001; 132: 891-899.
33. Orth-Gomér K, Halberg F, Sothern R, Akerstedt T, Theorell T, Cornélissen G. The circadian rhythm of ventricular arrhythmias. In: Takahashi R, Halberg F, Walker C, editors. Toward Chronopharmacology, Proc. 8th IUPHAR Cong. and Sat. Symposia, Nagasaki, July 27-28, 1981. Oxford/New York: Pergamon Press, 1982; 191-202.
34. Cornélissen G, Bakken E, Delmore P, Orth-Gomér K, Akerstedt T, Carandente O, Carandente F, Halberg F. From various kinds of heart rate variability to chronocardiology. Am J Cardiol 1990; 66: 863-868.
35. Huikuri HV, Kessler KM, Terracall E, Castellanos A, Linnaluoto MK, Myerburg RJ. Reproducibility and circadian rhythm of heart rate variability in healthy subjects. Am J Cardiol 1990; 65: 391-393.
36. Halberg F, Breus TK, Cornélissen G, Bingham C, Hillman DC, Rigatuso J, Delmore P, Bakken E, International Womb-to-Tomb Chronome Initiative Group: Chronobiology in space. Keynote, 37th Ann. Mtg. Japan Soc. for Aerospace and Environmental Medicine, Nagoya, Japan, November 8-9, 1991. University of Minnesota/Medtronic Chronobiology Seminar Series, #1, December 1991, 21 pp. of text, 70 ?gures.
37. Cornélissen G, Wendt HW, Guillaume F, Bingham C, Halberg F, Breus TK, Rapoport S, Komarov F. Disturbances of the interplanetary magnetic ?eld and human pathology. Chronobiologia 1994; 21: 151-154.
38. Cornélissen G, Halberg F, Wendt HW, Bingham C, Sothern RB, Haus E, Kleitman E, Kleitman N, Revilla MA, Revilla M Jr, Breus TK, Pimenov K, Grigoriev AE, Mitish MD, Yatsyk GV, Syutkina EV. Resonance of about-weekly human heart rate rhythm with solar activity change. Biologia (Bratislava) 1996; 51: 749-756.
39. Otsuka K, Cornélissen G, Halberg F. Circadian rhythmic fractal scaling of heart rate variability in health and coronary artery disease. Clinical Cardiology 1997; 20: 631-638.
40. Baevsky RM, Petrov VM, Cornélissen G, Halberg F, Orth-Gomér K, Akerstedt T, Otsuka K, Breus T, Siegelova J, Dusek J, Fiser B. Meta-analyzed heart rate variability, exposure to geomagnetic storms, and the risk of ischemic heart disease. Scripta medica 1997; 70: 199-204.

41. Otsuka K, Yamanaka T, Cornélissen G, Breus T, Chibisov SM, Baevsky R, Halberg F, Siegelova J, Fiser B. Altered chronome of heart rate variability during span of high magnetic activity. Scripta medica (Brno) 2000; 73: 111-116.
42. Halberg F, Cornélissen G, Otsuka K, Watanabe Y, Katinas GS, Burioka N, Delyukov A, Gorgo Y, Zhao ZY, Weydahl A, Sothern RB, Siegelova J, Fiser B, Dusek J, Syutkina EV, Perfetto F, Tarquini R, Singh RB, Rhees B, Lofstrom D, Lofstrom P, Johnson PWC, Schwartzkopff O, International BIOCOS Study Group. Cross-spectrally coherent ~10.5- and 21-year biological and physical cycles, magnetic storms and myocardial infarctions. Neuroendocrinol Lett 2000; 21: 233-258.
43. Halberg F, Cornélissen G, Schack B. Self-experimentation on chronomes, time structures; chronomics for health surveillance and science: also transdisciplinary civic duty? Behavioral and Brain Sciences, http://www.bbsonline.org/Preprints/Roberts/Commentators/Halberg.html
44. Cornélissen G, Otsuka K, Halberg F. Circadian Hyper-Amplitude-Tension (CHAT), a decreased (under-threshold) heart rate variability (DHRV) and an elevated pulse pressure (PP) are separate aspects of a chronomic cardiovascular disease risk syndrome. Abstract, 5th Japanese Annual Conference on Chronocardiology and Hypertension, Sept. 12, 2003, Sapporo, Japan. Session (3) Ambulatory BP, 14.

FRANZ HALBERG: AN ALLO-(AUTO)BIOGRAPHY

Othild Schwarzkopff, Germaine Cornélissen, Mary Sampson
WITH CONSULTATION BY **George Katinas (and Franz Halberg)**

Halberg Chronobiology Center
University of Minnesota
420 Delaware Street SE
Minneapolis, MN 55455, USA
Tel 612-624-6976, Fax 612-624-9989
Email: halbe001@umn.edu

Support:
U.S. National Institutes of Health (GM-13981) (FH),
Dr h.c. (mult) Earl E Bakken Fund (GC, FH),
University of Minnesota Supercomputing Institute (GC, FH)

The senior author looks back for more than half a century when Franz Halberg's professional life in the USA began and then took off to reach worldwide recognition within less than a decade after his arrival in the USA (in October 1948 at Harvard). In 1957, at the Semmering in Austria, he presided over an international conference of the International Society for the Study of Biological Rhythms (SBR), now the International Society for Chronobiology. He would preside over many more conferences on very many topics including but not limited to cardiology, computers in bioscience, human reproduction, oncology and pediatrics and special meetings arranged by national academies, the World Health Organization or the International Society for Research on Civilization Diseases and the Environment, of which he was U.S. vice-president (after he was offered and refused the international presidency). In fact, as president of the SBR for well over a decade, he brought about the above-noted name change to indicate the larger scope than mere rhythms for those dealing with chronomes (time structures) (1). He proposed chronobiology as a science in its own right, as historians recorded it, against opposition, as he does now. In 1983 (2), the social historians Alberto Cambrosio and Peter

Keating wrote:

The opposition of many biologists to the creation of a chronobiological discipline centred mainly on what was perceived as an attempt to occupy any area where time would play a role in biological analysis. According to [Colin] Pittendrigh, the matter can be first of all framed as a question of terms: 'The term "chronobiology" is something I oppose because it is unnecessary, pretentious, and inaccurate'. [Pittendrigh CS, personal communication to Cambrosio and Keating, 22 Jan 1981, note 20] And, more importantly, as there is not necessarily any common mechanism underlying rhythmic phenomena, then there is a still greater reason for opposing the disciplinarization of chronobiology: ... I had reached this position already in 1965 when the proposal arose to create a society for the study of the phenomena. It seemed to me that this would be comparable to some physiologist like Bernard suggesting that there ought to be a society for the study of homeostasis. [Pittendrigh to Cambrosio and Keating, 22 January 1981]

After having been refused the collaboration of researchers such as Pittendrigh and Aschoff in his initial 1964 project for the creation of chronobiological societies and journals, Halberg finally accepted the presidency of the SBR, then in full decline. In the years that followed, with the help of chronobiologists like Lawrence E. Scheving, Professor of Anatomy in Little Rock, Arkansas, and future secretary-treasurer of the International Society for Chronobiology, Halberg undertook the reconstruction of the SBR.

Franz held the presidency of the society for well over a decade; but in the course of the ensuing years, this did not prevent a series of contributions recorded, as of mid-September 2004, in 2,877 published titles. His publications and co-authors form a self-explanatory account of his life, along with views expressed by his late wife Erna (3), his close associate (4), Jürgen Aschoff (5), John E Pauly and Lawrence E Scheving (6), and himself (7). His bibliography is available on his website (http://www.msi.umn.edu/~halberg/), up to the time when he started to publish "Season's Appreciations", each including added bibliographies, and each summarizing his contributions beyond those in an Introduction to Chronobiology (8).

His intense interest and drive, bordering on obsession1, let him delve into the mysteries of the human body and its workings and he used his time in the US well. In 1948, when rocket scientists were brought to the US from Germany, Franz was brought from Austria for his promise in medicine, being transplanted from the Medical Faculty of the University of Innsbruck to Harvard Medical School and the Peter Bent Brigham Hospital in Boston. There, he started counting eosinophil cells in mouse blood in a research project to test for activity such as that of hormones of the adrenal cortex, at a time when no chemical or immunoassay was yet available. It was already known that adrenocortical hormones and epinephrine depressed eosinophil counts. But Franz could not confirm the hypothesis underlying an epinephrine test for adrenocortical function: the eosinophil count decreased every day on its own, which fact Franz eventually used as an internal bioassay in humans (9), and as an external one in the laboratory (10). For the latter bioassay, to cope with variability, Franz stayed up around the clock and stipulated in print that a check for eosinopenia with 2.8 mg of a test substance had to be carried out at 24 as well as 10 hours after implantation of the test substance. A few years later, he found effects with the same count in g, when he accounted for the rhythm (11). Franz's results were replicated within the next few years (12)2.

Because Franz went to bed much later than most, he had additional information and his results and views were at variance with the experience of all the other researchers in the same department of medicine. Franz's fellowship was terminated and he left Harvard.

In parting, the late George W. Thorn, the chief of the department, told Franz (using a colloquial American expression) that he admired Franz's "sticking to his guns", but added that everyone else could not be wrong while Franz was right. Within a year or so, Franz was vindictated and the epinephrine test was withdrawn.

Franz landed in 1949 under the wing of Prof. Maurice B. Visscher, head of the physiology department at the University of Minnesota, whom Franz had met while still at the University of Innsbruck. Franz has remained in Minnesota ever since and by 1955, eventually with formal affiliations in many departments of the university, including physiology, cancer biology, laboratory medicine, oral medicine and bioengineering, with still broader cooperation in the departments of classics, music and physical education.

As the titles of his publications over his first decade in Minnesota show, he did a great deal of eosinophil-counting and eventually cell division-counting. Dr. Dennis Lofstrom, a former student and now colleague, recalled that Franz in one run counted eosinophil cells without sleep for a week and, in between counts, briefly played tennis, his favorite sport (in which he was University of Minnesota faculty champion). The hypothalamic-pituitary-adrenal network was one of his early main topics. In 2003, Franz was invited by a new Journal of Circadian Rhythms to publish a description of his early scientific endeavors and academic development (7). Here and elsewhere, "daily variations", "24-hour rhythm", "24-hour scale", "phase relations of 24-hour periodicities", "cycling", "time dimension", "periodicity", "periodicity analysis", "time relations" and "photo-periodism" appear frequently in whatever was being studied in bacteria, eukaryotic unicells, a variety of insects, crayfish, catfish, a variety of stocks of mice (diurnal and nocturnal), rats (normo- and hypertensive), hamsters, monkeys and humans. The common denominator of these comparative studies across diverse species in vitro and in vivo time series gave rise eventually to the integrated rules of photo- and magneto , among other periodisms, as reciprocal spectra in and around us.

The measurements required new methods of data collection, and he became a pioneer in using telemetry in preparation for a Biosatellite project. Starting with periodograms, by the early 1950s Franz also led the development of methods in data analysis, which in turn led to biospectroscopy, for which he equated the computer with a prism. Instead of Frauenhofer lines, he identified innumerable bands as new facts, eventually with a new terminology. In his research projects, however, an emphasis on time structure is noticeable from the very start, enabled by the availability by the hundreds of John J. Bittner's (the discoverer of the first mammary cancer virus) carefully inbred strains of mice, differing in color from white over yellow and brown to black, perhaps more than the peas in Gregor Mendel's pea patch at the monastery in Brno. Just as the pea patch was the nursery of genetics, so was Bittner's mouse lab the cradle of chronobiology.

While early in his work Franz pursued the "clock", by 1951 he had found it in the adrenal cortex (13-15) and then pointed to another in the hypothalamus (14), an inference that others who found the suprachiasmatic nuclei called "visionary". But soon, he also found a clock in the cell (7, 8). The notion of pervasive ubiquitous "circadian" systems was first suggested in a paper published in 1959 by Franz in a review about 24-hour periodicity (15). In the part of this paper devoted to the discussion of definitions, he proposed it and "dian" as replacements for an earlier suggestion of "diel" and "dieloid". "Circadian" was officially accepted by the Committee of Nomenclature of the Society for Biological Rhythms, which Franz chaired, and thereafter by a nomenclature committee of the International Union of Physiological Sciences, of which he was a member, nominated by the late Nathaniel Kleitman. In 1960 "circadian" appeared in the title of the paper:

"Circadian performance rhythm in men adapting to an 8-hour day" (16). "Circadian" is a household word today and is rarely confused with "cicada" (the insects that in the eastern US emerge cyclically to reproduce, e.g., every 17 years). This possibility was considered in early discussions (perhaps in 1951) of the term with the late Henry Nash Smith, in his time the foremost scholar in American Studies and Franz's lifelong friend and mentor, who polished Franz's English while Franz in turn looked after the health of the Smith family in Minnesota, before Henry accepted the chairmanship of the English department of the University of California at Berkeley. But their cooperation continued.

In this first decade Franz's conviction about the utter importance to include timing as the indispensable control into all testing and/or assessment of physiological variables resulted first from the large yet predictable extent of 24-hour change in blood cell counts, i.e., its genetically conserved variability among inbred strains of mice. The confusion that can be resolved only by mapping cycles when phase-shifts and phase-drifts characterize one of two groups being compared was one of the experiences that set his course for future years and decades. Opposite results could be had only as a function of timing in concurrent studies (7). Franz compared a group of mice with a very high rate of breast cancer feeding in the evening with an ovariectomized group feeding in the morning, with a very low breast cancer incidence. The former group, with a low breast cancer incidence, happened to be phase-shifted by meal timing, insofar as the restricted diet, for the laboratory assistant's convenience, was given in the morning. Franz solved this puzzle, which determined the subsequent course of his life: he found a statistically significant difference in one direction (a seemingly beautiful discovery) in the first study, no difference in a second study carried out earlier in the day, and the opposite result at a third time, getting up earlier and earlier for each subsequent study (7). He realized (before publication!) that he had compared a phase-shifted group fed a calorie-restricted diet in the morning in light, with a group feeding in the dark; this contributed to the contrasting result showing that one group was higher at one time and the reverse was true about 12 hours later. He still wonders why rhythms are not **the** indispensable control.

The methodological importance of rhythms as **the** control information was reinforced by his finding of a desynchronized rhythm in mice without eyes, so that by comparing the same sham-operated and eyeless groups, he found them to be different at one time but not at another. This was another hint of endogenicity, called by his friend Earl E. Bakken (of pacemaker and Medtronic fame) "free-running", another concept that in chronobiology has become a household word. The most dramatic finding was that the same stimulus, first noise, then X-ray, and eventually drugs, could kill or be survived in the same dose, only as a function of timing, a topic to be amplified later. It culminated in Franz's development of what his close associate in research Germaine Cornélissen has called "chronotheranostics", derived from chronos, time, therapy, and diagnostics (based on marker rhythms). Chronotherapy, originating in the laboratory, from studies on mice susceptible to audiogenic convulsions, was thus the second empirical challenge, derived from the fact that life or death could result from the identical stimulus only as a function of timing.

The third and major finding was his discovery of rhythms at the cellular level that were logical in view of earlier findings by others on cycles in mitotic activity, but nonetheless surprising when nucleic acid was regarded as the organism's most constant feature. The idea of a cyclic RNA or DNA formation was considered so outlandish that Franz's request for a technician was refused. To his good luck, however, Cyrus P. Barnum, the senior professor in this aspect of biochemistry, gave him the benefit of the doubt and told him "I'll

be your technician". Barnum was soon fascinated, and Franz did a great deal of mapping with him on the liver and brain; they discovered rhythms in the formation of RNA and DNA and found that the formation of the former preceded that of the latter, probably the first hint that an RNA-world preceded ours, and certainly at odds with the still-prevailing linear dogma that information flows from DNA => RNA => protein. These findings were and probably still are the bases for a pioneering enterprise with the number of dedicated researchers in the field increasing, as apparent from well over 9,000 citations of Franz's work.

We also see a gradual widening of the scope and extent of his interests. After studies of the "circadians" we see the gradual inclusion of the "circaseptans", followed by the further extension of the "circa-system" to circasemiannuals, -annuals, cisannuals, neartransannuals, fartransannuals, decadals and -multidecadals. These are all being mapped with their characteristics and their uncertainties. The provision of 95% confidence intervals of the periods as well as amplitudes, phases and, when possible and acceptable, of the waveform has become indispensable. Variables are not only cyclic but also spectral in the sense that they undergo cycles with many frequencies that all relate to multiple environmental frequencies. Hence, nothing is ever totally deterministic. Feed-sidewards (a concept also coined by Franz based on extensive data obtained first with Salvador Sanchez de la Pena) relate to all physiological and many other processes. By 1969, Franz showed (7) that not only have different variables related, for instance, to the brain, heart or hormones, different characteristic frequencies, as seen in EEGs, ECGs or time series of metabolite excretion, but these same frequencies are shared by more than one variable, and that the timing of different variables can differ so that in caves, subjective time estimation may differ from environmental time and other organismic or variable-related internal time scales - relativistic biologic time.

While others modeled theoretical multi-oscillator systems, Franz decided to map the real oscillations so that, in at least some variables, there is a now-known spectrum of various components with different frequencies. To do this mapping, Franz had to develop methods. In a paper entitled "Chronobiology", the study of temporal characteristics of biologic phenomena, he proposed and documented an objective description of biological time structure. It was published in 1969 in the journal Annual Review of Physiology (17) and has been recognized by Current Contents as a "Citation Classic". It was also the first time the term "chronobiology" officially appeared, by invitation, in a major official review journal, thanks to another lifelong friend, the late Arthur Giese, Professor of Biological Sciences at Stanford and a world authority on marine biology. Today chronobiology is, far too slowly, on the path toward being a science in its own right, and Franz now nurses chronomics, the cartography of time structures, Scheme 1 (1).

Franz's current hobby-horse, in addition to many other different research fields, is blood pressure chronomics, and for good reason. Helped by rapidly advancing computer technology and eventually a total dependence on it, at first with his cosinor method he pleads for longitudinal nonstop surveillance, preferably automatically and ambulatorily with an as-one-goes inferential statistical interpretation. For this variable, "the microscopy of time series" can be introduced into everyday health care. "The endpoints of rhythms are to represent an objective, numerical and inferential statistical aspect of physiological variables in making the diagnosis, in deciding on the timing of treatment and in checking its effects".

In long blood pressure series, the characteristics of the timing of the recurrence of high values can be determined as period and phase objectively, rather than by eyeballing, each

endpoint with a measure of its uncertainty (its 95% confidence interval). Likewise, the extent of change is gauged objectively as the amplitude of an oscillation, whereby excessive oscillations sound an alarm to do something about a well demonstrated risk of very unpleasant cardiac events. The undue swing of blood pressure, among other criteria of variability, notably when it remains in a range of acceptable values, would be recognized neither by the conventional single measurement spotcheck, nor by the (false) gold standard of a chronobiologically uninterpreted 24-hour profile.

The last decade was also governed by a bold foray into the physics of the natural sciences: a new chronocosmobiophysics. The ability to measure the extent of our dependence on the environment and trying to quantify it is the *Leitmotiv* of Franz's work within the last decade. Kp, aa, Dst, Schwabe, Wolf and Hale's polarity changes, solar wind speed and, certainly, cosmic rays, all have, albeit confounded signatures in our physiology (18). The importance of the unseen aspects of the sun was long known in Russia (19-23), albeit without the information on new cycles and the invariably indispensable provision of inferential statistical uncertainties associated with new and old cycles. The latter inferential biospectroscopy and bio-cross-spectroscopy remains in the foreground of discussions and calculations. After all, humans developed under the visible guiding light of the sun and also under the auspices of non-photic, non-thermic aspects of environments driving us from near and far. It is astonishing how far and how close we are in pitch with the all pervasive and ubiquitous periodicities, or rather broader time structures, of our terrestrial and extraterrestrial surroundings, which cannot be resolved by eyeballing. Examples showing the merit of time-microscopy abound, some of them in the appendix following this text.

The most rewarding and readily immediately usable timing is in the field of blood pressure disorders. Monitors and analyses are available for a scientific approach in diagnostics and therapeutics based on timing. Franz is implementing such chronotheranostics on himself as a model for the use of chronomics in vascular disease prevention, i.e., prehabilitation, and complementing rehabilitation. Altered variability that represents a very high risk of severe disease (Figure 1) must be assessed and treated, notably in areas with limited resources that cannot readily afford coronary artery bypass grafting. The reduction of a very high risk of vascular disease is a challenge similar to that of vaccination in dealing with infectious diseases (24). Moreover, by monitoring one may not only contribute to self-health care but also importantly to science (25).

As to the adjective "unnecessary" (cf. 2) concerning chronobiology, cancer chronotherapy and prehabilitation for stroke prevention may eventually provide answers. As to "inaccurate", there is a choice between Franz's time-microscopy with point-and-interval estimates underlying each statement vs. eyeballing. Eyeballing means necessarily selection. In the laboratory, one can focus on a "good" animal and discard "bad" records from "rogues". But one cannot build a biology dealing only with non-rogues. Rogues then remain unevaluated. When a patient happens to have a rogue's record, that patient's sibling may help by providing a transplant, but the sibling cannot substitute for the diagnostic procedure. In most medical research, it has become indispensable to use inferential statistics. One must not dispense with numerical statistical hypothesis testing and parameter estimation and with the uncertainties involved in the N-of-1 endeavor of any one pertinent aspect in clinical practice: computer-implemented "inaccurate" chronobiology and chronomics could and should eventually fill this void in everyday self-help in health care, thus detecting alterations in dynamics that eyeballing may miss and cannot quantify even where noted.

As to "pretentious", Franz's humility has been emphasized elsewhere (4) and is further obvious from his happily providing his critic Colin Pittendrigh upon the latter's request with a no-longer "inaccurate" and "unnecessary" phase chart, which the critic (apparently happily) included in the final paper he published before he died (26). Franz in turn dedicated his own biography (7) to his critics, thereby recognizing that they were eloquent popularizers of the field, not only to Pittendrigh but also to Jürgen Aschoff. Aschoff honored Franz in print (5), showing Franz's photograph following likenesses of Claude Bernard, Charles Edouard Brown-Séquard, Arnold Adolf Berthold, Ernest Henry Starling, Archilochus, Hippocrates, Aristotle, Galen, Christoph Wilhelm Hufeland and Julien-Joseph Virey as Franz's intellectual ancestors. Aschoff finished the sequence with another portrayal of Archilochus, reading his poetry to women, as Franz was often accompanied by his wife Erna. Aschoff then proclaimed Franz a master of two fields, endocrinology and chronobiology.

Incidentally, Aschoff begins the sequence of Franz's ancestors with the same Claude Bernard to whom Pittendrigh alluded (2) when he implied (in a letter to two philosophers which might not have been intended for publication) that Bernard might have suggested "that there ought to be a society for the study of homeostasis". Conceivably, Pittendrigh also might not have known that before he turned to the constancy of the internal environment, Bernard, at the peak of his career, named the *variabilité énorme du milieu intérieur* as one of his two major discoveries (27). As apparent from Table 1, Franz has devoted his lifetime to the debunking of homeostasis that draws a curtain of ignorance over the range of everyday physiology, justifying baselines consisting of time-unqualified spotchecks, the essence of clinical and laboratory medicine today. *Ceterum censeo, homeostasim esse delendam.*

EPILOGUE

The search for mechanisms led Franz first to the adrenal cortex, then to the pituitary and the hypothalamus, and soon to a clock in each cell. But there is more than just a "clock" -- more than circadians -- in the cell. Each cell also contains other parts of chronomes -- other rhythms, trends and chaos -- and the mechanism acting on them is found outside the cell in the cosmos in both its photic and non-photic reciprocal cycles that eventually made it into the genome. *Omnis rhythmus, omnis cyclus e cosmo.* This led to a new periodic system with some not-so-old photic components circadians and circannuals and some older (in physics) half-yearly, about 10.5-yearly and about 21-yearly cycles and brand new 1.05-year cycles. The biological transyears were discovered in keeping with the view of reciprocal spectra in and around us. First, the physicists found the about 1.3-year as an oscillation in the solar wind (28); soon, physicists added the 1.5-1.7-year components (29). But lessons about neartransyears learned in unicells and babies, in phylogeny and ontogeny, reinforced the search and the finding in the Minnesota chronomic as well as chronobiological center of near-transyears of the solar wind and geomagnetism.

Moreover, these physical features were traced back far beyond the decades of satellite recordings of solar wind speed, beyond the about 1.3 hundred years of geomagnetic recordings and beyond the history of only hundreds of years of sunspots and auroral appearances. Like Gilbert with his book on magnetism, Franz with his contributions on a spectrum of natural photic and nonphotic cycles, first circadians and circannuals, then many cycles in magnetism, tries to find some answers for the way the physical environment, unseen as well as seen, acts upon us, in certain frequency windows, wobbly

ones, spectral regions, rather than wide bands and certainly not Frauenhofer-like lines. But in a finite record, these are relatively well-defined finite regions such as between 1.0 and 1.2 years for a neartransyear and between 1.2 and 2.0 years for Richardson's (28), Mursula and Zieger's (29), and other fartransyears (24, 25).

Many more components now in the solar wind may be identified as counterparts in the dynamic living fossils that constitute organisms. Even if some life forms changed more than others, they may all show vestiges informing us about past physical environments that preceded the emergence of physics by a billion years. Biologists showed that the emergence of leading physicists also shows a cycle (30). By aligning biological and physical time series, the study of chronomes can trace the latter through evolution. Life can help physics as physics helped life. But Franz strives for more than such interdisciplinary endeavors that have just yielded transyears and neartransyears.

By examining the transdisciplinary effects of our environment, from economics to international battles, chronomics may provide a basis for a better understanding of societal diseases of war and crime. This endeavor was approached by many, except it was dealt with as if the periodicities involved were spectral lines a la Frauenhofer, which they certainly are not. The alternative attitude had been that such changes are unpredictable. The truth lies in between, in the spectra that consist of photic and nonphotic cycles in the time structures of both photo- and magnetoperiodism, and in how, what must not be ignored (at specific frequencies), drummers, from near and far, influence us, through the adrenal, pituitary, hypothalamic and pineal network, Franz's original love. Working with Salvador Sanchez de la Pena, Franz has described multifrequency interactions within the network and with the environment as feedsidewards.

These replacements of time-unqualified feedbacks and feedforwards by feed-sidewards -- time-qualified many-way internal and/or external interactions -- may well be the mechanisms that, via chronobiology and chronomics, may lead us from the physical world; from the biosphere; and from the noosphere, in this technological age of the mind (= *noos*), to the ethosphere, the sphere of good and bad behaviors. This is why Franz stays in his study at the university for long hours and on long weekends and looks at precisely the mechanisms that lead back to adrenals and other endocrines and to the neurons that interact with them in certain windows of magnetic frequencies. In this context, Franz attempts to transplant the endocrinologist's remove-and-replace approach, from the gland removed by a surgeon and the replacement of a hormone, on the one hand, to the frequencies removed and replaced by the sun, on the other hand, to test associations of magnetic cycles with the ethosphere.

On the personal side, Franz is known for his congenial attitude to co-workers at all levels, in helping to bring them to the forefront while he stands back. His deeply felt wish to be a positive influence in the world we live in and in the world to come emphasizes the improvement of both preventive individual and societal care by inclusion of the all-pervasive time factor in testing results and recognizing and treating monitoring-revealed risk rather than only disease. His impeccable workmanship in his research includes himself as a subject whenever possible. All this is embedded in his character.

He has not yet taken time to write books in science and has refused extremely lucrative offers to popularize his findings and his ideas about the importance of timing of all physiological processes. He chose instead to persist in the nitty-gritty day-to-day work of his research. Bittner's mouse laboratories were the "cradle of chronobiology", where he first secured evidence of a genetically inborn time-dependence of physiological processes. Now, his current virtual laboratory is a transdisciplinary world. Franz does not seek the

limelight, but continues to accumulate evidence for a time structure-based combination of a chronodiagnosis and chronotherapy of elevated disease risk, of great importance in its own right, while the same chronomes may be critical for advancing gene-based diagnosis and gene therapy. I conclude with Franz's phrasing of his outlook into the future: "And so the learning goes on the battle against cancer, against stroke and most important against diseases of society. For all of these endeavors, chronobiologists should be a united family everywhere". There is no surrender to the more leisurely way of life in the waning years of his career. The real issue is the control in whatever we do: cycles with their uncertainties. Only then can science serve the ethosphere. There can be no alternative to a chronobiology- and chronomics-based chronobioethics. But to show its promise in this all-important task and to obtain the necessary means, chronomics has to prove itself in cancer treatment (Appendix 1), in general transdisciplinary scientific method and concept (Table 1) and in stroke prevention (5, 19, 31; see also 32). John E Pauly and Lawrence E Scheving wrote in 1987: "Although Franz Halberg probably spends as much time at work as any living scientist, he still finds time to be a devoted husband and father and a wonderful friend. Chronobiology is always with him, and those who know him well can recall discussing experimental procedures on the tennis court or novel statistical analyses on the ski slopes. ... The exploration of many of the basic phenomena in chronobiology and many of the methods for their quantitative study are inseparably tied to the name and the work of Franz Halberg. These volumes are dedicated to him in recognition of his outstanding contributions to science, his pioneering efforts in the field of chronobiology, and the help and encouragement he has provided colleagues all over the world." (6). So are these lines a quarter century later, while Franz continues in real and figurative tennis and still tries to return every real and virtual "ball".

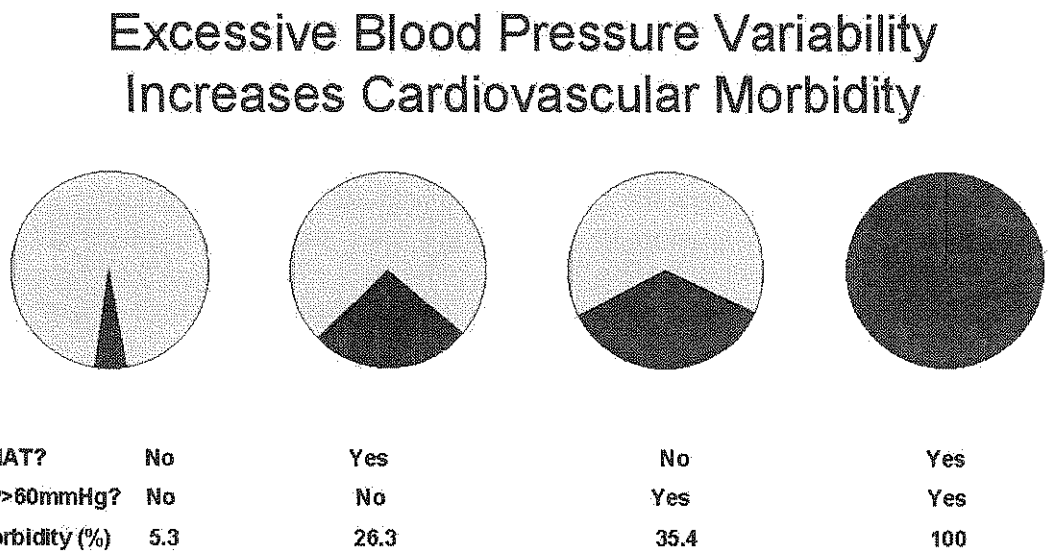
REFERENCES

1. Halberg F, Cornélissen G, Otsuka K, Schwartzkopff O, Halberg J, Bakken EE. Chronomics. Biomedicine and Pharmacotherapy 2001; 55 (Suppl 1): 153-190.
2. Cambrosio A, Keating P. The disciplinary stake: the case of chronobiology. Social Studies of Science 1983; 13: 323-353.
3. Halberg E. 39 years of fun and excitement. Chronobiologia 1989; 16: iii-vi.
4. Cornélissen G, Halberg E, Halberg Francine, et al. Chronobiology: a frontier in biology and medicine. Chronobiologia 1989; 16: 383-408.
5. Aschoff J. Speech after dinner. In: Aschoff J, Ceresa F, Halberg F, editors. Chronobiological Aspects of Endocrinology. Stuttgart: F.K. Schattauer Verlag; 1974/Chronobiologia 1974; 1 (Suppl. 1): 483-495.
6. Pauly JE, Scheving LE. Dedication. Progress in Clin and Biol Res 1987; 227A: xxiii-xxvii.
7. Halberg Franz, Cornélissen G, Katinas G, et al. Transdisciplinary unifying implications of circadian findings in the 1950s. J Circadian Rhythms 2003; 1: 2. 61 pp. www.JCircadianRhythms.com/content/pdf/1740-3391/1/2.pdf
8. Cornélissen G, Halberg F. Introduction to Chronobiology. Medtronic Chronobiology Seminar #7, April 1994, 52 pp. (Library of Congress Catalog Card #94-060580; http://www.msi.umn.edu/~halberg/).
9. Halberg F, Cohen SL, Flink EB. Two new tools for the diagnosis of adrenal cortical dysfunction. J Lab Clin Med 1951; 38: 817.
10. Halberg F. Some correlations between chemical structure and maximal eosinopenia in adrenalectomized and hypophysectomized mice. J Pharmacol Exp Ther 1952; 106: 135-140.
11. Halberg F. Some physiological and clinical aspects of 24-hour periodicity. J Lancet 1953; 73: 20-32.
12. Brown HE, Dougherty TF. The diurnal variation of blood leucocytes in normal and adrenalectomized mice. Endocrinology 1956; 58: 136-148.
13. Halberg F, Visscher MB, Flink EB, Berge K, Bock F. Diurnal rhythmic changes in blood eosinophil levels in health and in certain diseases. Journal-Lancet (Minneapolis) 1951; 71: 312-319.
14. Halberg F, Halberg E, Barnum CP, Bittner JJ. Physiologic 24-hour periodicity in human beings and mice, the lighting regimen and daily routine. In: Withrow RB, editor. Photoperiodism and Related Phenomena in Plants and Animals. Ed. Publ. No. 55. Washington DC: AAAS; 1959. p. 803-878.
15. Halberg F. Physiologic 24-hour periodicity; general and procedural considerations with reference to the adrenal cycle. Z. Vitamin-, Hormon-u Fermentforsch 1959; 10: 225-296.
16. Hauty GT, Steinkamp GR, Hawkins WR, Halberg F. Circadian performance rhythms in men adapting to an 8-hr day. Fed Proc 1960; 19: 54.
17. Halberg F. Chronobiology. Annu Rev Physiol 1969; 31: 675-725.
18. Halberg F, Cornélissen G, Otsuka K, et al. Chronoastrobiology: proposal, nine conferences, heliogeomagnetics, transyears, near-weeks, near-decades, phylogenetic and ontogenetic memories. Biomed Pharmacother, in press.
19. Chizhevsky AL. Action de l'ionisation de l'atmosphere et de l'ionisation artificielle de l'air sur les organismes sains et les organismes malades. In: Piéry M, ed. Traité de Climatologie: Biologique et médicale. Tome premier. Paris: Masson et Cie; 1934. p. 662-673.

20. Sigel F. Vinovato solntse [The Sun is Guilty]. (Dreier W, Lerche D, German translation; Göring H, scientific editor, German edition.) Moscow: Verlag MIR/Leipzig: VEB Fachbuchverlag, 1975.
21. Breus TK, Chibisov SM, Baevsky RM, Schebzuhov KV. The chronostructure of heart biorhythms under effects of external environment factors. Moscow: Russian University of People's Friendship, Polygraph Service; 2002. 231 pp.
22. Breus TK, Rapoport SI. Magnitnye bure: Medico-biologicheskie i geofizicheskie aspektii. Moscow: Izdatel'stvo Sovetskii Sport; 2003. 192 pp.
23. Masalov AV, Syutkina EV. Magnetic storms and neonatal blood pressure and heart rate chronomes. Neuroendocrinol Lett 2003; 24 (Suppl 1): 111-116.
24. Cornélissen G, Halberg F, Bakken E, et al. 100 or 30 years after Janeway or Bartter, Healthwatch helps avoid "flying blind". Biomed Pharmacother, in press.
25. Halberg F, Cornélissen G, Schack B. Self-experimentation on chronomes, time structures; chronomics for health surveillance and science: also transdisciplinary civic duty? Behavioral and Brain Sciences, http://www.bbsonline.org/Preprints/Roberts/Commentators/Halberg.html
26. Pittendrigh CS. Temporal organization: reflections of a Darwinian clock-watcher. Ann Rev Physiol 1993; 55: 17-54.
27. Bernard C. De la diversité des animaux soumis a l'experimentation. De la variabilité des conditions organiques dans lesquells ils s'offrent a l'expérimentateur. J. de l'Anatomie et de la Physiologie normales et pathologiques de l'homme et des animaux 1865; 2: 497-506.
28. Richardson JD, Paularena KI, Belcher JW, Lazarus AJ. Solar wind oscillations with a 1.3-year period. Geophys Res Lett 1994; 21: 1559-1560.
29. Mursula K, Zieger B. The 1.3-year variation in solar wind speed and geomagnetic activity. Adv Space Res 2000; 25: 1939-1942.
30. Verhulst J, Onghena P. Periodic birth-year pattern of the founders of quantum physics. Psychological Reports 1996; 78: 19-25.
31. Cornélissen G. Time structures (chronomes) in us and around us: a tribute to Franz Halberg. This volume.
32. Regal PJ. Astronomy and medicine: Chronobiology and Franz halberg. This volume.

Legends

Figure 1. Altered blood pressure (BP) dynamics (chronomics) raise the risk of cardiovascular morbidity from 5.3% to 100% (25).



Footnotes

1. The double entendre of *possessed* is the contribution solely of the senior author, at variance with her co-authors, and remains included as another sign of Franz's humility (GC). For an alternate interpretation, see (31, 32; see also 5, 6).
2. "For many years conflicting opinions have appeared in the literature concerning cyclic variation of the circulating leucocytes. Recently, a diurnal variation of large magnitude has been well established for the circulating eosinophils in a variety of animals, Halberg and Visscher (1950), Halberg (1953), Halberg, Visscher and Bittner (1953), ..." (12).

Appendix 1: Toward chronotherapy or rather chronotheranostics: From survival or death because of timing a stimulus or drug

Research on susceptibility to noise showed that, dependent only on the timing of the stimulus administered to susceptible mice, the animals survived or died (A1). The same difference between death and survival was thereafter found for drugs and X-rays. Eventually, cancer radiotherapy became a role model for chronobiological clinical pharmacotherapy with chronodiagnostics consisting of the use of a marker rhythm, namely core temperature. In patients with very advanced perioral cancers, accessible tumor temperature was measured around the clock for days as a marker of dynamics:

Different groups of patients were then randomly assigned to treatment either at peak tumor temperature, 4 or 8 hours before or 4 or 8 hours after peak tumor temperature, or "as usual", i.e., without a systematic scheduling by reference to the tumor's temperature profile, Figure A1. After treatment, patients were rated according to tumor size semi-quantitatively.

Of 40 timed-treatment patients, 8 in each of the 5 timed groups, and of 10 patients treated as usual, Figure A2, shows that those treated at peak tumor temperature had the fastest tumor regression (left) and the best therapeutic result (right). The improvement in outcome was statistically significant. Timing can be as much or more important than is any other choice with a given treatment kind, complementing considerations of dosage. Multiple marker rhythms can reveal different optimal times for specific goals of lowering toxicity and raising efficacy and for a triangulation of timing among these goals (A2).

The need for servicing immobile instrumentation in special facilities by special staff during times other than regular hours was and is an obstacle encountered in implementing chronoradiotherapy. No such obstacles are encountered in many cases of marker rhythm-guided drug therapy, which can be self-administered by the patient, as in the case of blood pressure disorders. When self-treatment at home is not possible and care providers are not flexible, the patient can adjust his/her schedule to that of the staff and can validate this adjustment by self-monitoring. Thus, when a former president of the American Statistical Association needed cancer chemotherapy at times different from the provider's office hours, he was sufficiently motivated to change his sleep-wake schedule and to monitor his temperature rhythm. He was then treated at the best circadian time estimated by temperature rhythmometry. He lived for years thereafter and died of a vascular disease.

REFERENCES

A1. Halberg F, Cornélissen G, Wang ZR, et al. Chronomics: circadian and circaseptan timing of radiotherapy, drugs, calories, perhaps nutriceuticals and beyond. J Exp Therapeutics Oncol 2003; 3: 223-260.

A2. Halberg Francine, Halberg J, Halberg E, Halberg Franz. Chronobiology, radiobiology and steps toward the timing of cancer radiotherapy. In: Goldson AL, volume editor. Cancer Growth and Progression, vol. 9, ch. 19, Kaiser H, series editor. Dordrecht: Kluwer Academic Publ.; 1989. p. 227-253.

Legends

Figure A1. Circadian stage-dependence of tumor regression on the timing of irradiation by reference to the time of peak tumor temperature.

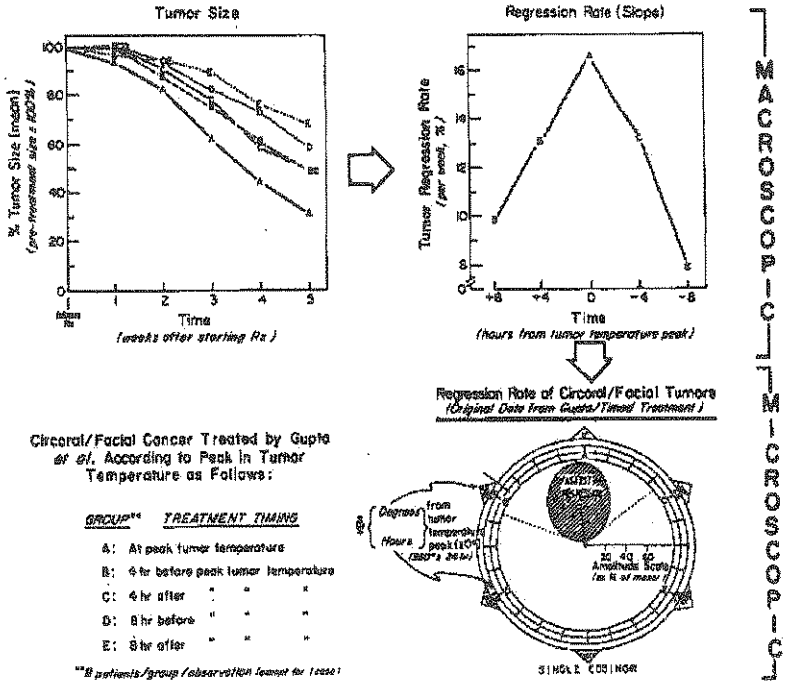
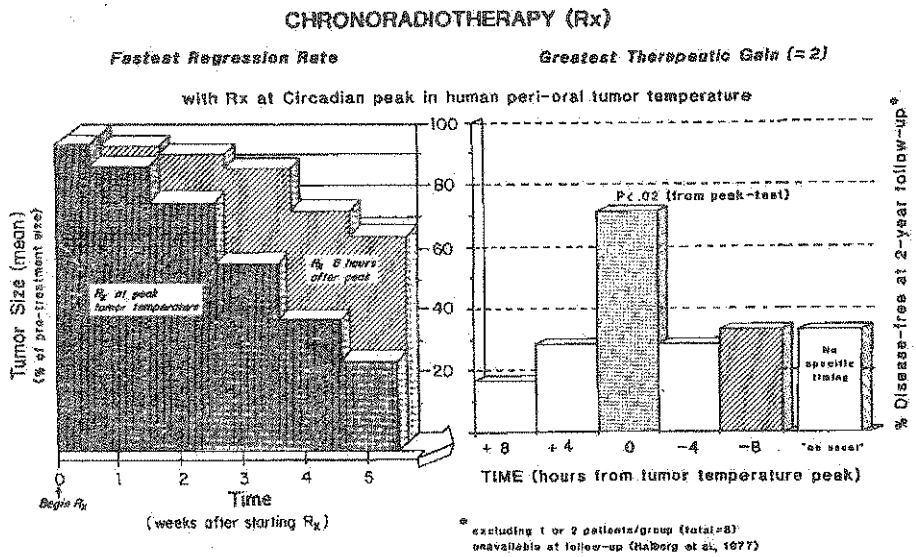
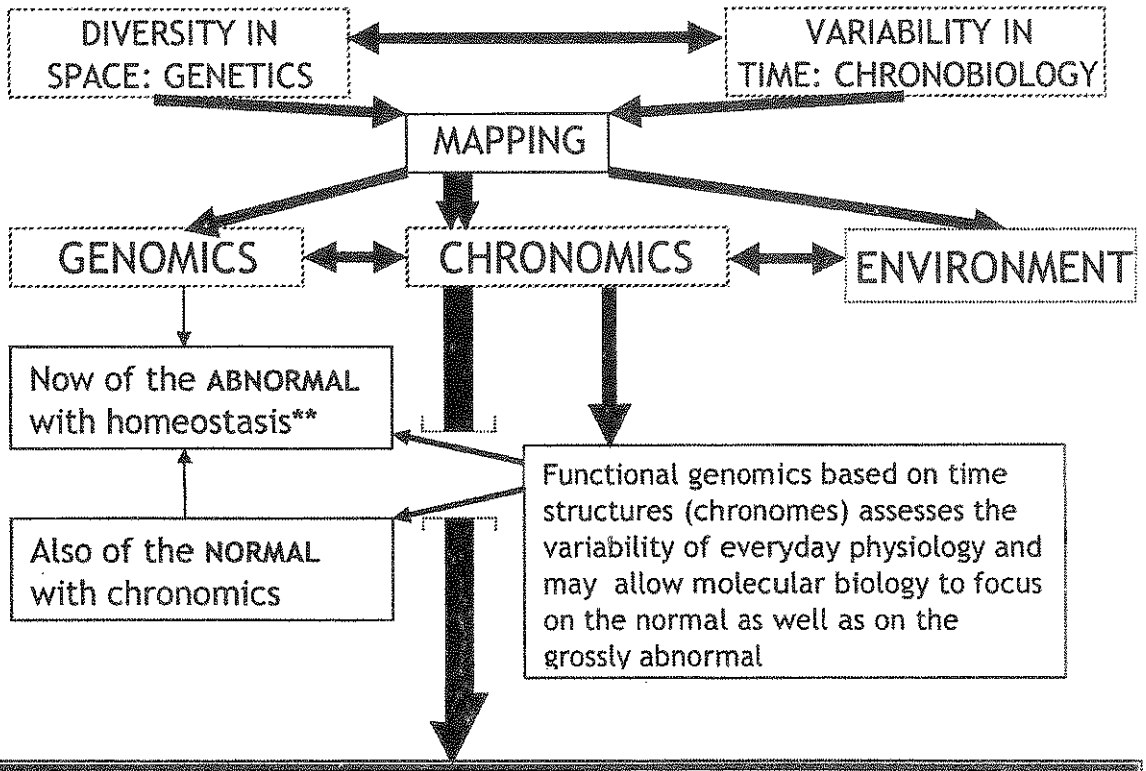


Figure 14.39. Chronoradiotherapy of oral cancers referred to tumor surface temperature as a tumor marker rhythm and evaluated by a short term therapeutic endpoint.

Figure A2. Treatment at the time of peak tumor temperature is associated with a faster tumor regression and with a better disease-free survival at a 2-year follow-up.



Imaging biological structural diversities in space-time for alignment with complementary environmental structures*



The major merit of chronomics in its own right is the quantification of normalcy and the detection of earliest risk elevation, shadowing meritorious current, only circadian approaches to jet lag, shift-work and even the addition by timed treatment of years to a cancer patient's (partly high quality) life, achievements second only to prevention: PRE-habilitation rather than only REhabilitation, the basis of a timely & timed treatment of risk elevation for health

*An outcome of puzzles encountered in the 1950s (Halberg F, Visscher MB. Proc Soc exp Biol [N.Y.] 1950; 75: 846-847); more in Halberg F et al., J Circadian Rhythms, http://www.jcircadianrhythms.com/

**Based on usually time-, gender-, age-, geography-, ethnicity- and social class-unspecified normal ranges that constitute the curtain of ignorance drawn over everyday physiology by homeostatic baselines, lifted by imaging time structures of rhythms with frequencies covering over 10 orders of magnitude in and around us

Table 1: Why chronomics -- mapping variability and its mechanisms in and around us

View of:	I. HOMEOSTASIS: Response physiology outside the normal range	II. CHRONOMICS: Everyday physiology in the normal range	UTILITY OF II
1. DEFINITION OF NORMALCY, e.g., HEALTH	**NEGATIVE:** ABSENCE OF ABNORMALITY, e.g., OF SIGNS, SYMPTOMS, BIOCHEMICAL ABNORMALITY OR DISEASE[a]	**POSITIVE:** BY ENDPOINTS FROM PARAMETRIC AND NONPARAMETRIC ASSESSMENT OF RULES IN VARIABILITY AND CORRESPONDING RANGES OF ACCEPTABILITY	**TIME STRUCTURE** IS **THE** CONTROL IN WHATEVER WE DO, REPLACING TIME-UNQUALIFIED BASELINES BASED ON SPOTCHECKS
2. ENDPOINTS	• **ORIGINAL VALUES:** CASUAL MEASUREMENTS AT TIMES OF CONVENIENCE, NOT NECESSARILY OF PERTINENCE (e.g., OF "THE" BLOOD PRESSURE WITH >40% UNCERTAINTY IN DIAGNOSIS IN CASES OF BORDERLINE HYPERTENSION): TIME-UNSPECIFIED: • MEAN • STANDARD ERROR • DAY-NIGHT RHYTHMS	**ENDPOINTS IN CHRONOMES[b]:** TIME-CODED: • ORIGINAL VALUES • STANDARD DEVIATIONS (e.g., 6-h, 24-h) • MESOR(s), M • AMPLITUDE(s), A • ACROPHASE(s), ϕ • PERIODS, τ • WAVEFORM(s) (A, ϕ) • TRENDS • CHAOTIC ENDPOINTS • RESIDUALS	• PROVIDES INFORMATION IN CHRONOBIOLOGIC SOFTWARE FOR QUANTIFYING HEALTH • GUIDES TIMED TREATMENT THAT, e.g., HAS GREATLY PROLONGED THE SURVIVAL OF CANCER PATIENTS • VALIDATES TREATMENT EFFECTS OF HARMONICS
3. QUANTIFICATION OF NORMALCY, e.g., HEALTH	**POPULATION-BASED:** PERCENT ABNORMALITY, e.g., MORBIDITY AND MORTALITY	**INDIVIDUALIZED ENDPOINTS:** P-VALUES FOR STATISTICAL SIGNIFICANCE AND FOR SCIENTIFIC (e.g., CLINICAL) SIGNIFICATION OF ENDPOINTS OF VARIABILITY	RECOGNIZING RISK ELEVATION OR OTHER ABNORMALITY BEFORE OVERT DISEASE

4. INTERPRETATION OF REALITY	**HOMEOSTASIS:** NO NEED TO MEASURE MORE THAN SPOTCHECKS. PUTATIVE (IMAGINARY) SET POINTS	**CHRONOMES** IN TIME SERIES: CONSISTING OF a) RHYTHMS, b) TRENDS, c) DETERMINISTIC AND OTHER CHAOS, d) ANY RESIDUALS AND INTERACTIONS AMONG a, b, c AND d	**DETECTING CHRONOME ALTERATIONS:** 1) CIRCADIAN OVERSWINGING OF BLOOD PRESSURE OR 2) DEFICIENT HEART RATE VARIABILITY OR 3) ABOVE-THRESHOLD PULSE PRESSURE 4) ALTERED ABOUT-YEARLY RHYTHMS IN CIRCULATING PROLACTIN AND TSH SIGNALING BREAST AND PROSTATIC CANCER RISK ELEVATION
5. ATTITUDE TOWARD VARIABILITY	**FOE:** IGNORED OR VIEWED AS A CONFOUNDER	**FRIEND:** OF INTEREST IN ITS OWN RIGHT	AS A **TOOL** AND **SOURCE OF INFORMATION**[c]
6. BIOSYSTEMS' BEHAVIOR IF PERTURBED	SETTLING DOWN TO A **STEADY STATE** (CONSTANCY) OR LIMITED RANDOM "HUNTING", e.g., AS (MISTAKENLY ANTICIPATED) WHEN A SINGLE BLOOD PRESSURE IS TAKEN AFTER SOME (≤30) MINUTES OF REST	**DYNAMIC CHRONOMES** THAT CHARACTERIZE HEALTH WITHIN CHRONOBIOLOGIC LIMITS SET BY THE INTERMODULATION OF THE CHRONOMES' SPONTANEOUS (α)-, REACTIVE (β)-, AND ONE-WAY (γ)- OR n-WAY MODULATING (δ) RHYTHMS	**POSITIVE INDIVIDUALIZED QUANTIFICATION OF HEALTH**
7. ANALOGY	**THERMOSTATS** WITH "HUNTING" NOISE	**PENDULUMS** IN RESOLVABLE CHRONOMES	**PREDICTION**
8. PHYSIOLOGIC OR NORMAL RANGES OF VARIATION	BROAD, INDIVISIBLE; EQUATED TO RANDOM **NOISE** CURRENT STANDARD FOR DIAGNOSIS AND TREATMENT	STRUCTURED, PREDICTABLE[a]; RESOLVED INTO REFERENCE RANGES **(CHRONODESMS)** FOR ENDPOINTS OF CHRONOMES	CIRCADIAN BLOOD PRESSURE (BP) AMPLITUDE OR CIRCADIAN STANDARD DEVIATION (SD), e.g., FOR DETECTING EFFECT OF IN UTERO EXPOSURE TO BETAMIMETICS

9. ACTION?	**ELIMINATION OF A CONFOUNDER** (RHYTHM): IMPOSSIBLE TO IMPLEMENT; INCOMPATIBLE WITH DETECTION OF CIRCADIAN BLOOD PRESSURE DISORDER COMPATIBLE WITH DIAGNOSING THE SAME PERSON AS NORMOTENSIVE IN THE MORNING AND HYPERTENSIVE IN THE AFTERNOON	MONITORING AND AS-ONE-GOES ANALYSES, AND, ON THIS BASIS, THERAPEUTIC **ACTION** IF AND ONLY IF AND WHEN NECESSARY	DETECTS TREATABLE OVERSWINGING OF BP-A, WHICH CARRIES A 720% INCREASE IN RISK OF ISCHEMIC STROKE; IMPROVES CANCER TREATMENT
10. SOURCES OF VARIATION	**EXOGENOUS** RESPONSES TO STIMULI FROM PROXIMITY MOSTLY FROM THE HABITAT NICHE	**ENDOGENOUS** AND **EXOGENOUS**: RESPONSES TO STIMULI FROM NEAR **AND FAR**, INCLUDING COSMOS	RESOLUTION OF IMPACT OF STORMS IN SPACE ON MYOCARDIAL INFARCTIONS ON EARTH: NEED FOR A **SPACE WEATHER REPORT ?**[e]
11. MECHANISM	**FEEDBACKS** ALONG AXES OF TIME-UNQUALIFIED "MODULATION" THAT IS EXPECTED TO ACT AT ANY TIME LIKE THE deus ex machina IN A PHYSIOLOGICAL TRAGEDY SINCE OUTCOMES MAY IN FACT BE UNPREDICTABLE	**FEEDSIDEWARDS**, TIME-QUALIFIED MANY-WAY INTERNAL AND/OR EXTERNAL INTERACTIONS IN NETWORKS WITH ALTERNATING OUTCOMES WHICH ARE PREDICTABLE (INSOFAR AS THEY RHYTHMICALLY RECUR) AS A CHRONOMODULATION	PREDICTABLE SINCE RHYTHMIC NEURO-ENDOCRINO-VASCULAR INTERMODULATIONS CAN ACCOUNT FOR OUTCOMES THAT MAY BE AS DIFFERENT AS STIMULATION VS. INHIBITION OF IMMUNITY
12. HIERARCHY	**UP/DOWN**	**COLLATERAL:** ALTERNATING PRIMACY AMONG INTERMODULATING MULTI-FREQUENCY RHYTHMS IN CHRONOMES	FOCUSING ON SELECTED TASKS AT DIFFERENT TIMES
13. TELEONOMY	**RIGHTING** AND **REGULATION**	ANTICIPATORY, PREPARATORY **COORDINATION**	GREATER **FLEXIBILITY**
14. SIMPLIFIED ANALOGY	THERMOSTAT	PENDULUM	—

15. BIOLOGIC EVOLUTION	DARWINIAN, EXTERNALLY **ADAPTIVE**	MORE AND MORE INTERNAL AND **INTEGRATIVE** WHILE EXTERNALLY ADAPTIVE TO BOTH NATURE AND NURTURE	INSTRUMENTED SELF-HELP TOWARD A SCIENTIFIC APPROACH TO ETHICS
16. HEALTH AND ENVIRONMENTAL CARE	MEDICAL TREATMENT OFTEN LIMITED AND LATE, GIVEN MOSTLY AFTER THE DIAGNOSIS OF OVERT DISEASE[e]	OPTIMIZATION ACCORDING TO MARKER CHRONOMES (OF INTERVENTIONS BY DRUGS AND/OR DEVICES, e.g., PACEMAKERS, WITH DIAGNOSIS AND TREATMENT REFINED BY NARROWED REFERENCE RANGE AND ASSESSMENT WITHIN THAT RANGE OF CHRONORISK LEADING TO PREVENTIVE TREATMENT TIMED BY MARKER RHYTHMS (THAT ALSO SERVE TO VALIDATE EFFECT)	e.g., CATASTROPHIC AND IATROGENIC DISEASE PREVENTION
17. ANIMAL HUSBANDRY, APICULTURE, AQUACULTURE AND ECONOMIC ENTOMOLOGY	CONVENIENCE	CHRONOME-BASED[f]	**OPTIMIZATION:** GREATER EFFICACY; FEWER UNDESIRABLE EFFECTS
18. VALUE	OFTEN WASTEFUL	COST-EFFECTIVE	WASTE REDUCED
19. SEEKING INANIMATE AND ANIMATE ORIGINS	STRATIGRAPHY FOR IDENTIFYING, IN GEOLOGICALLY ANALYZED **SPACE**, SEQUENCES IN **TIME**; RADIOCARBON DATING	ADDITIONAL TRACING OF CHRONOMO-ONTOGENY AND CHRONOMO-PHYLOGENY[g] IN THE CONTEXT OF GLIMPSES OF CYCLES IN CORRESPONDING SPANS OF A FIGURATIVE COSMO-ONTOGENY	ADDS TO KNOWLEDGE OF THE PAST TO BETTER OPTIMIZE THE FUTURE

20. LIFE IN THE SCHEME OF PHYSICAL AND CULTURAL THINGS	SURVIVAL OF THE FITTEST WITH HUMANS DOMINATING FOOD CHAINS VIEWED IN THE PERSPECTIVE OF BIOENERGETICS IN A **MOSTLY TERRESTRIAL ECOLOGY**	PHYSICALLY AND SOCIALLY CHRONOMODULATING AND THUS INFORMATIVELY AND INTEGRATIVELY EVOLVING BIOTA MOLDED BY HUMAN CULTURE; homo NOT ONLY faber BUT cosmoinformans AND chronomodulans IN A **BUDDING BROAD CHRONOCOSMO-ECOLOGY**[h]	HUMANS SAFEGUARD THE INTEGRITY OF THE BIOSPHERE AS IT EXTENDS INTO THE COSMOS AND AS WE SPECULATIVELY YET BY JOINING THE APPROACHES BY ABLATIONS, SUPERPOSED EPOCHS AND RESONANCE TESTS CONCOMITANTLY EXPLORE THE TEMPORAL ASPECTS OF OUR ORIGINS, POSSIBLY REPRESENTED BY OUR CHRONOMES THAT IN TURN MAY REFLECT A LONG-PAST ENVIRONMENT
21. INVESTIGATOR SATISFACTION	**FRUSTRATING WORK** WHEN (WITHOUT SPECIFICATION OF CHRONOBIOLOGIC TIMING, EVEN AT THE SAME CLOCK-HOURS) ONE GETS CONFUSING AND/OR OBSCURING, EVEN OPPOSITE RESULTS FROM THE SAME INTERVENTION	**SHEER FUN:** LONG-STANDING CONTROVERSY IS RESOLVED BY ACCOUNTING FOR BOTH THE GENETIC AND BROADLY ENVIRONMENTAL BASES OF THE FEEDSIDEWARDS AMONG INANIMATE AND ANIMATE CYCLES THAT CONSTITUTE LIFE; DISEASE RISK RECOGNITION PROMISES TO LEAD TO THE PREVENTION OR TIMED TREATMENT OF CATASTROPHIC DISEASES SUCH AS STROKE, CANCER OR SUDDEN DEATH	**INCREASED PRODUCTIVITY**

Just as contemporary physics, by fission and fusion, gathers more and more energy by splitting the atom, biomedicine gathers more and more information by splitting the normal value range into time structures, thereby resolving, e.g., rhythms (fission) and looking at their feedsideward interrelations (fusion) for a better understanding of an interdigitated, indivisible Janus-faced inseparable soma and psyche.

[a]Health promotion is a step in the right direction, by its recommendations of attention to diet, exercise or relaxation, as long as it is then followed by a chronobiologic assessment of the effect of recommended procedures, rather than merely by the old reliance of ruling out the occurrence of values outside the normal range.

[b]Location and dispersion indices include the determination from histogram of values, of means (arithmetic, geometric, harmonic), median, mode, minimum, maximum, 100% and 90% ranges, interquartile range, standard deviation, standard error; these endpoints are computed from time-unspecified single values in the context of the homeostatic approach, whereas in the chronobiologic framework the location and dispersion indices are used as such on time-specified samples and on time series-derived parameters, i.e., on each of the endpoints (chrones: M, A, ϕ, [A_n, ϕ_n], etc.) of the chronomes.

[c]An international womb-to-tomb chronome initiative with aims primarily at stroke and other catastrophic vascular disease prevention, by focus as a start upon chronocardiology in general and blood pressure and heart rate dynamics in particular. Those interested may consult the chronobiology home page on the Web at http://www.msi.umn.edu/~halberg/

[d]Information from the physiologic range for prevention, diagnosis or treatment is much refined when this range is individualized and interepreted in the light of a personalized background as well as in the context of gender-, age-, ethnicity- and chronome stage-specification.

[e]The need for forecasting storms in space should be explored further on the basis of systematic studies aligning physiological lifetime monitoring and clinical and archival statistical studies with ongoing physical data collection near and far, both for ascertaining effects and in studying countermeasures. Blood pressure, heart rate and other physiological and psychological monitoring would also provide basic information on any cross-spectral and other associations (feedsidewards) within and among biological and environmental chronomes while further providing reference values of medical interest.

[f]Even if some preventive measures have also been long implemented, e.g., by vaccination, and even if recently more and more hygienic measures (such as exercise and caloric, fat and sodium restriction) are also popular, all can be greatly improved by timing designed with chronobiologic individualization. The alternative, current action based on group results, its

unquestionable overall merits notwithstanding, fails to recognize, for instance, that the blood pressure response to salt may differ as a function of circadian stage, and there are indeed individuals in whom the addition of salt lowers rather than raises blood pressure.

[g]Even after the death of a cockroach, when bacteria take over, periodicities (e.g., in oxygen consumption) may not be "eliminated", but continue with increased amplitude. Critical information may be lost by filtering variation deemed to be undesirable since it lies beyond one's conventional scope.

[h]Development from the egg of rhythms (some may be much older than shards) and of other constituents of chronomes to trace their homeo- or heterochronically roughly "recapitulatory" development across species, with both ontogeny and phylogeny, perhaps tracing in their turn the concomitant development of the geocosmic environment. This distant basic goal can be pursued with the immediate reward of obtaining indispensable reference values for the diagnosis of two chronobiologic risk syndromes, circadian hyperamplitudetension, briefly CHAT, and a deficient heart rate variability, briefly CAHRVs, just as an extreme deficit in heart rate jitter associated with an increase in the risk of ischemic stroke or of a myocardial infarction of 720 and 550%, respectively.

FRANZ HALBERG AND NEUROIMMUNOMODULATION

Novera Herbert Spector
International Institute for NIM Research
4014-G Layang Circle
Carlsbad, CA 92008, USA

I am honored to be asked to add my tribute to this Festschrift for Professor Halberg. Franz Halberg is one of the (few) giants of science of the twentieth century.

I first met Franz 35 years ago, when I was Chief of Neurophysiology at the Walter Reed Institute of Research. I was immediately impressed by his breadth of knowledge, his absolutely straightforward (but always courteous) manner, and his unswerving devotion to science, scientific research, and humanity.

Since then my respect for him has constantly grown. He has spawned an ocean of new ideas, even coined the terminology for one of the most important fields of biomedical research. He inspired and taught others in many countries around the world to collaborate and to produce mountains of invaluable data. His own exemplary research, along with his students and colleagues, most especially the great Germaine Cornélissen, covers every area of the *practice and theory of chronobiology*, and inevitably flows over to many other domains of biomedical research.

Dr. Cornélissen has asked me to mention Franz's contributions to one of my own favorite fields, neuroimmunomodulation (NIM). NIM deals with the 3-way interactions among the nervous, endocrine, and immune systems. (For a slightly expanded definition and very brief history and further references on this subject, see Spector NH [1999]. Neuroimmunomodulation, in *Encyclopedia of Neuroscience*, 2nd ed.)

The first "official" symposium on NIM was held at the International Congress of Physiological Sciences in Budapest, Hungary, in 1981. At this congress and at many subsequent formal and informal symposia on NIM, Franz was an invited speaker and enthusiastic participant, as usual (for Franz) contributing many novel ideas and offering excellent research data.

As early as 1955, Halberg (et al.) was publishing papers on immune function which change daily in a rhythmic fashion (Halberg et al., 1955; Wrba et al., 1985). Later with Cornélissen, they introduced the term *chrononeuroimmunomodulation*, stressing the periodicity of neural, endocrine, and immune functions. He and Cornélissen were co-authors of the first review of interactions of NIM and the environment (Spector et al., 1995).

One of the most important teachings of Dr. Halberg to the medical professions, which he has been preaching emphatically for as long as I can remember, has still not penetrated into the resistant dogma and daily practice of most physicians. This lesson is the dire warning: A medication prescribed for the ailing patient, administered in the evening, *may have an opposite effect* from the desired one when administered in the morning (Wrba et al., 1989)! (We need hardly add the also too-often ignored reminder to the practitioner:

that three green pills *per diem* to the 250-pound 20-year-old male cannot be the correct dosage for the 100-pound 80-year-old female!)

The teachings of Franz Halberg and his colleagues with regard to chronobiology have already had a profound effect upon the healing professions. Let us hope that in the 21st century his voice will be heard to an even greater extent.

REFERENCES

The following partial list of contributions of Halberg and colleagues to immunology and NIM was assembled by Germaine Cornélissen (many thanks!):

1. Carandente F, Angeli A, DeVecchi A, Dammacco F, Halberg F. Multifrequency rhythms of immunological functions. (Chronoimmunomodulation by an Antimicrobial Agent, 1989, F. Carandente, F. Dammacco, F. Halberg, eds.) Chronobiologia 1988; 15: 7-23.
2. Carandente F, Dammacco F, Halberg F. Preface. (Chronoimmunomodulation by an Antimicrobial Agent, 1989, F. Carandente, F. Dammacco, F. Halberg, eds.) Chronobiologia 1988; 15: 3-4.
3. Carandente F, DeVecchi A, Halberg F, Cornélissen G, Dammacco F. Toward a chronoimmunomodulation by cefodizime in multiple myeloma and chronic uremia. (Chronoimmunomodulation by an Antimicrobial Agent, 1989, F. Carandente, F. Dammacco, F. Halberg, eds.) Chronobiologia 1988; 15: 61-85.
4. Cavallini M, Magnus C, Halberg F, Florack G, Sutherland DER. Murine model for a chronoimmunotherapy of allo-transplantation: circadian and circannual variation in cyclosporine effects. Europ Surg Res 1983; 15 (Suppl. 1): 19-20.
5. Cavallini M, Magnus G, Halberg F, Liu T, Field MY, Sibley R, Najarian JS, Sutherland DER. Benefit from circadian timing of cyclosporine revealed by delay of rejection of murine heart allograft. Transplant Proc 1983; 15 (#4, Suppl. 1): 2960-2966.
6. Cecchettin M, Sanchez de la Pena S, Halberg F, Cornélissen G, Ungar F, Brown G, Mantero F, Vecsei P, Simmons D, Walker RF, Scheving LE. Reproducibility of pineal feed-sideward upon the ACTH 1-17 effect on adrenocortical function. In: Neuroimmunomodulation, Proc. 1st Int. Workshop on NIM, Bethesda, MD, Nov. 27-30, 1984. Bethesda: IWGN; 1985. p. 139-141.
7. Chihara G, Carandente F, Halberg F. Chronoimmunology and temporal aspects of lentinan effect upon LOU rat immunocytoma growth. Proc. AACR/ASCO 1979; 20: 111, abstract 450.
8. Cornélissen G, Halberg E, Halberg Francine, Halberg J, Sampson M, Hillman D, Nelson W, Sánchez de la Pena S, Wu J, Delmore P, Marques N, Marques MD, Fernandez JR, Hermida RC, Guillaume F, Carandente F. Chronobiology: a frontier in biology and medicine. Chronobiologia 1989; 16: 383-408.
9. Cornélissen G, Halberg F. Chrononeuroimmunomodulation: need for standardization and assessment of a broad spectral structure. Abstract #342, 1st International Congress, ISNIM (4th Int. Workshop on Neuroimmunomodulation), Florence, May 23-26, 1990. p. 457.
10. Cornélissen G, Halberg F. Toward a "chron-sensus" on neuroimmunomodulation, with "modulation" operationally and inferentially defined. In: Fabris N, Jankovic BD, Markovic BM, Spector NH eds Ontogenetic and Phylogenetic Mechanisms of

Neuroimmunomodulation: From molecular biology to psychosocial sciences. Ann NY Acad Med Sci, 1992; 650: 60-67.
11. Cornélissen G, Halberg J, Johnson D, Rigatuso J, Sitka U, Syutkina EV, Grigoriev AE, Halberg F. About-weekly (circaseptan) versus about-daily (circadian) prominence in prematures and adults. Abstract, Perspectives in Immunology and Medicine 1944-1994: A Symposium in Honor of Robert A. Good, St. Petersburg, Florida, May 20-21, 1994.
12. Cornélissen G, Sánchez de la Pena S, Halberg F. From drug synergism and antagonism to endocrine chronoimmunomodulators. J Interdiscipl Cycle Res 1985; 16: 245-246.
13. DeLisi C, Hiernaux J, Halberg F. Circaseptan component of mammalian antibody production in response to immunization. Chronobiologia 1983; 10: 119.
14. Ertel RJ, Halberg F, Ungar F. Circadian system phase-dependent toxicity and other effects of methopyrapone (SU-4885) in mice. J Pharmacol exp Ther 1964; 146, 395-399.
15. Fernandes G, Halberg F, Yunis E. Circadian variations in immune response of mice. In: McGovern J, Reinberg A, Smolensky M, editors. Chronobiology in Allergy and Immunology. Springfield, Ill.: Charles C. Thomas; 1977. p. 233-249.
16. Fernandes G, Halberg F, Yunis E, Good RA. Circadian rhythmic plaque-forming cell response of spleens from mice immunized with SRBC. J Immunol 1976; 117: 962-966.
17. Fernandes G, Yunis EJ, Halberg F, Good RA. Circadian rhythm in antibody-forming cells from spleens of BALB/c mice immunized against sheep red blood cells (SRBC). Chronobiologia 1975; 2 (Suppl. 1): 20-21.
18. Fernandes G, Yunis EJ, Nelson W, Halberg F. Differences in immune response of mice to sheep red blood cells as a function of circadian phase. In: Scheving LE, Halberg F, Pauly JE eds. Chronobiology, Proc. Int. Soc. for the Study of Biological Rhythms, Little Rock, Ark. Stuttgart: Georg Thieme Publishers/Tokyo: Igaku Shoin Ltd.; 1974. p. 329-338.
19. Halberg E, Cornélissen G, Bakken E, Halberg F. Chrononeuroimmunomodulation (chrono-NIM): lead-lag cross-correlations of mental state and tumor burden. Abstract, 2nd Int. Cong. Int. Soc. Neuroimmunomodulation, Paestum (Salerno), Italy, September 12-17, 1993. Chronobiologia 1994; 21: 144-145.
20. Halberg E, Cornélissen G, Haus E, Fine RL, Walker R, Von Hoff D, Halberg Francine, Halberg J, Halberg Franz. Ampli?cation [on comments by Berry DA. Power of chronobiologic pilots: a statistician's opinion. Chronobiologia 1993; 20: 213-214]. Chronobiologia 1993; 20: 214-218.
21. Halberg E, Halberg F. Chronobiologic study design in everyday life, clinic and laboratory. Chronobiologia 1980; 7: 95-120.
22. Halberg E, Sanchez de la Pena S, Halberg F. Host and tumor temperature, motor activity and cyclosporine (Cs) immunopharmacology. Biological Rhythms and Medications, Proc. 1st Montreux Conf. Chronopharmacol., Montreux, Switzerland, 1984, #303.
23. Halberg F. Some physiological and clinical aspects of 24-hour periodicity. Journal-Lancet (Minneapolis) 1953; 73: 20-32.
24. Halberg F. Symposium on "Some current research methods and results with special reference to the central nervous system." Physiopathologic approach. Amer J ment Defic 1960; 65, 156-171.

25. Halberg F. Temporal coordination of physiologic function. Cold Spr Harb Symp quant Biol 1960; 25: 289-310.
26. Halberg F. Organisms as circadian systems; temporal analysis of their physiologic and pathologic reponses, including injury and death. In: Symposium on Medical Aspects of Stress in the Military Climate, Walter Reed Army Institute of Research (Col. William D. Tigertt, Medical Corps, Director and Commandant), Walter Reed Army Medical Center (Maj. Gen. A.L. Tynes, Medical Corps, Commanding), Washington DC, 22-24 April 1964. Washington DC: U.S. Government Printing Office 1965-778-714; 1965. p. 1-36.
27. Halberg F. Chronobiology: methodological problems. Acta med rom 1980; 18: 399-440.
28. Halberg F. Report on radioimmunoassay 1980: a conference held in Gardone Riviera, May 8-10, 1980. Soc ital Biochim clin 1980; 6-7: 389-399.
29. Halberg F. *Quo vadis* basic and clinical chronobiology: promise for health maintenance. Am J Anat 1983; 168: 543-594.
30. Halberg F. Can chronobiology be ignored in the treatment of immune deficiency and cancer ?. Cancer Det Prev 1987; 11: 64.
31. Halberg F, Barnum CP, Silber RH, Bittner JJ. 24-hour rhythms at several levels of integration in mice on different lighting regimens. Proc Soc exp Biol (NY) 1958; 97, 897-900.
32. 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 (NY) 1955; 88: 169-173.
33. Halberg F, Cornélissen G. About-7-day (circaseptan) pattern of DNA labelling under daily STH stimulation in mouse tissues: a metachronanalysis. Abstract, Perspectives in Immunology and Medicine 1944-1994: A Symposium in Honor of Robert A. Good, St. Petersburg, Florida, May 20-21, 1994.
34. Halberg F, Cornélissen G, Bingham C, Fujii S, Halberg E. From experimental units to unique experiments: chronobiologic pilots complement large trials. in vivo 1992; 6: 403-428.
35. Halberg F, Cornélissen G, Haus E, Lakatua D, Ikonomov O, Stoynev A. Hypothalamic transplantation for cancer prevention: a thought experiment with old data and new tools: marker chronomes. Abstract, 2nd Int. Cong. Int. Soc. Neuroimmunomodulation, Paestum (Salerno), Italy, September 12-17, 1993, p. 23.
36. Halberg F, Cornélissen G, Schwartzkopff O, Watanabe Y, Otsuka K. Feedsidewards: intermodulation (strictly) among time structures, chronomes, in and around us, and vasculo-neuroimmunity. Abstract A24, 4th Int. Cong. International Society for Neuroimmunomodulation, Lugano, Switzerland, September 29-October 2, 1999. Neuroimmunomodulation 1999; 6: 400.
37. Halberg F, Gupta BD, Haus E, Halberg E, Deka AC, Nelson W, Sothern RB, Cornélissen G, Lee JK, Lakatua DJ, Scheving LE, Burns ER. Steps toward a cancer chronopolytherapy. In: Proc. XIV International Congress of Therapeutics, Montpellier, France: L'Expansion Scienti?que Française; 1977. p. 151-196.
38. Halberg F, Halberg E, Barnum CP, Bittner JJ. Physiologic 24-hour periodicity in human beings and mice, the lighting regimen and daily routine. In: Withrow RB, editor. Photoperiodism and Related Phenomena in Plants and Animals. Ed. Publ. No. 55. Washington DC: AAAS; 1959. p. 803-878.
39. Halberg F, Haus E, Cardoso SS, Scheving LE, Kühl JFW, Shiotsuka R, Rosene G, Pauly JE, Runge W, Spalding JF, Lee JK, Good RA. Toward a chronotherapy of

neoplasia: Tolerance of treatment depends upon host rhythms. Experientia (Basel) 1973; 29: 909-934.
40. Halberg F, Haus E, Halberg E, Berg H, Cornélissen G. Sphygmo-neuroimmunomodulation (S-NIM): multifrequency feedsidewards among the circulation, cancer and melatonin. Abstract, 2nd Int. Cong. Int. Soc. Neuroimmunomodulation, Paestum (Salerno), Italy, September 12-17, 1993. Chronobiologia 1994; 21: 143.
41. Halberg F, Haus E, Kuzel M, Lakatua D, Kawasaki T, Ueno M, Uezono K, Omae T, Knapp E, Günther R. Cost-effective chronobiologic monitoring. In: Albertini A, Da Prada M, Peskar BA, editors. Radioimmunoassay of Drugs and Hormones in Cardiovascular Medicine. Amsterdam: Elsevier/North Holland Biomedical Press; 1979. p. 107-120.
42. Halberg F, Jacobson E, Wadsworth G, Bittner JJ. Audiogenic abnormality spectra, 24-hour periodicity and lighting. Science 1958; 128, 657-658.
43. Halberg F, Johnson EA, Brown BW, Bittner JJ. Susceptibility rhythm to E. coli endotoxin and bioassay. Proc Soc exp Biol (NY) 1960; 103, 142-144.
44. Halberg F, Levi F, Chihara G, Scheving LE. Chronobiologic intermodulation in immunomodulation and neurophysiology. Proc. XXVIII Int. Cong. Physiol. Sci., Budapest, Hungary, July 13-19, 1980. Chronobiologia 1980; 7: 134.
45. Halberg F, Nelson W, Cornélissen G, Haus E, Scheving LE, Good RA. On methods for testing and achieving cancer chronotherapy. Cancer Treatment Rep 1979; 63: 1428-1430.
46. Halberg F, Sonkowsky R, Delmore P, Spector NH, Raab F, Schaffer E, Cornélissen G. Tensions of everyday life: benetensive, transtensive or maletensive chronome-dependent outcomes of chrononeuroimmunomodulation. In: Cornélissen G., Halberg E., Bakken E., Delmore P., Halberg F. (eds.): Toward phase zero preclinical and clinical trials: chronobiologic designs and illustrative applications. Minneapolis: University of Minnesota Medtronic Chronobiology Seminar Series, #6, September 1992. p. 83-137.
47. Halberg F, Spink WW, Albrecht PG, Gully RJ. Resistance of mice to brucella somatic antigen, 24-hour periodicity and the adrenals. J. clin. Endocrinol. 1955; 15: 887.
48. Halberg F, Stephens AN. Susceptibility to ouabain and physiologic circadian periodicity. Proc. Minn. Acad. Sci. 1959; 27, 139-143.
49. Halberg F, Vecsei P, Wu J, Lange R, Ratajczak H, Thomas P, Hagen K, Robel P, Baulieu E-E, Cornélissen G. Nonsense correlations or neuroendocrine chronoimmunomodulation by dehydroepiandrosterone (DHEA) and its sulfate (S). Abstract #341, 1st International Congress, ISNIM (4th Int. Workshop on Neuroimmunomodulation), Florence, May 23-26, 1990, p. 456.
50. Halberg F, Visscher MB. Regular diurnal physiological variation in eosinophil levels in five stocks of mice. Proc Soc exp Biol (NY) 1950; 75: 846-847.
51. 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 51: 329-335, 1952.
52. Halberg F, Visscher MB, Bittner JJ. Eosinophil rhythm in mice: Range of occurrence; effects of illumination, feeding and adrenalectomy. Amer J Physiol 1953; 174: 109-122.
53. Halberg F, Visscher MB, Flink EB, Berge K, Bock F. Diurnal rhythmic changes in blood eosinophil levels in health and in certain diseases. J-Lancet (USA) 1951; 71: 312-319.

54. Haus E, Halberg F. 24-hour rhythm in susceptibility of C mice to a toxic dose of ethanol. J appl Physiol 1959; 14, 878-880.
55. Hayes DK, Shade L, Cornélissen G, Halberg E, Miller RW, Halberg F. Chronomodulatory infradian synchronization by *placebo* or ACTH 1-17 of *Musca autumnalis* mortality on shifted lighting regimens. Proc. 1st Int. Workshop on NIM, Bethesda, MD, Nov. 27-30, 1984, IWGN, Bethesda, 1985. Chronobiologia 1985; 12: 361-365.
56. Kanabrocki EL, Graham L, Veatch R, Greco J, Kaplan E, Nemchausky BA, Halberg F, Sothern R, Scheving LE, Pauly JE, Wetterberg L, Olwin J, Marks GE. Circadian variations in eleven radioimmunoassay variables in the serum of clinically healthy men. Progress in Clinical and Biological Research 1987; 227A: 317-327.
57. Kovats T, Nelson W, Berner D, Halberg F. Endotoxin chronotolerance. In: Reinberg A, Halberg F, editors. Chronopharmacology, Proc. Satellite Symp. 7th Int. Cong. Pharmacol., Paris 1978. Oxford/New York: Pergamon Press; 1979. p. 223-230.
58. Kuzel M, Zinneman H, Nelson W, Halberg F, Rosen G, Scheving LE, Haus E. Competing circadian effects of methylprednisolone and rat weight, light chains, immunocytoma size and survival. Chronobiologia 1978; 5: 295-311.
59. Lester B, Sánchez de la Pena S, Wu J, Halberg F. Chronoimmunomodulation by cefodizime (CDZ) of the susceptibility of $B_6D_2F_1$ mice to a melanoma B16 (MB16). Chronobiologia 1989; 16: 154.
60. Levi F, Halberg F, Chihara G, Byram J. Chronoimmunomodulation: circadian, circaseptan and circannual aspects of immunopotentiation or suppression with lentinan. In: Takahashi R, Halberg F, Walker C, eds. Toward Chronopharmacology, Proc. 8th IUPHAR Cong. and Sat. Symposia, Nagasaki, July 27-28, 1981. Oxford/New York: Pergamon Press; 1982. p. 289-311.
61. Levi F, Halberg F, Haus E, Sánchez de la Pena S, Sothern RB, Halberg E, Hrushesky W, Brown H, Scheving LE, Kennedy BJ. Synthetic adrenocorticotropin for optimizing murine circadian chronotolerance for adriamycin. Chronobiologia 1980; 7: 227-244.
62. Levi F, Hrushesky W, Haus E, Halberg F, Scheving LE, Kennedy BJ. Experimental chrono-oncology. In: Scheving LE, Halberg F, Pauly JE, eds. Chronobiology, Proc. Int. Soc. for the Study of Biological Rhythms, Little Rock, Ark. Stuttgart: Georg Thieme Publishers/Tokyo: Igaku Shoin Ltd.; 1974. p. 481-512.
63. Marques MD, Cornélissen G, Cutkomp LK, Halberg F, Marques N. Experimental models for the study of chronoimmunomodulation (rhythms of the springtail *Folsomia candida, Collembola: Isotomida)*. (Chronoimmunomodulation by an Antimicrobial Agent, 1989, F. Carandente, F. Dammacco, F. Halberg, eds.) Chronobiologia 1988; 15: 183-189.
64. Nelson W, Halberg F. Effects of a synchronizer phase-shift on circadian rhythms in response of mice to ethanol or ouabain. Space Life Sci 1973p;. 4: 249-257.
65. Panzenhagen H, Speirs R. Effect of horse serum, adrenal hormones, and histamine on the number of eosinophils in the blood and peritoneal fluid of mice. Blood 1953; 8: 536-544.
66. Ratajczak HV, Lange RW, Sothern RB, Hagen KL, Vecsei P, Wu JY, Halberg F, Thomas PT. Surgical influence on murine immunity and tumor growth: relationship of body temperature and hormones with splenocytes. Proc. Soc. exp. Biol. Med. 1992; 199: 432-440.
67. Roitman-Johnson B, Sothern RB, Halberg F, Blumenthal MN. Circadian immunologic rhythms and their implications in the diagnosis and treatment of atopic disorders. In:

Recent Advances in the Chronobiology of Allergy and Immunology, M.H. Smolensky, A. Reinberg, J.P. McGovern, eds., Pergamon Press, Oxford/New York, 1980, pp. 65-72.
68. Sanchez de la Pena S, Halberg E, Halberg F, Cornélissen G, Sothern RB, Yunis E. Circadian modulation of the anti-tumor effect of cyclosporine. In: Neuroimmunomodulation, Proc. 1st Int. Workshop on NIM, Bethesda, MD, Nov. 27-30, 1984, IWGN, Bethesda, 1985, pp. 252-257.
69. Sánchez de la Pena S, Halberg F, Ungar F, Lakatua D. Ex vivo hierarchy of circadian-infradian rhythmic pineal-pituitary-adrenal intermodulations in rodents. In: Pancheri P, Zichella L, eds. Biorhythms and Stress in the Physiopathology of Reproduction, New York: Hemisphere; 1988. p. 177-214.
70. Sánchez de la Pena S, Wu J, Halberg F, Cornélissen G, Wetterberg L, Halberg E, Lakatua D, Marques N, Harvey J, Bazin H, Tran B, Carandente F. Immunomodulation by an antibiotic in malaria and aging: internal endocrine schedule-shift and chronopharmacologic feedsidewards. (Chronoimmunomodulation by an Antimicrobial Agent, 1989, F. Carandente, F. Dammacco, F. Halberg, eds.) Chronobiologia 1988; 15: 129-182.
71. Schwartzkopff O, Cornélissen G, Halberg F. Immunologic, neurophysiologic and cosmologic cameos from Arrhenius and Prawdicz-Neminski in chronobiological perspective. In: Mikulecky M, ed. Chronobiology & Its Roots in the Cosmos. High Tatras, Slovakia, September 2-6, 1997. Bratislava: Slovak Medical Society; 1997. p. 37-42.
72. Spector NH, Dolina S, Cornélissen G, Halberg F, Markovic BM, Jankovic BD. Neuroimmunomodulation: neuroimmune interactions with the environment. In: Fregly MJ, Blatteis CM, editors. Handbook of Physiology, Section 4: Environmental Physiology. New York: American Physiological Society/Oxford University Press; 1995. p. 1537-1550.
73. Wrba H, Dutter A, Sánchez de la Pena S, Wu J, Carandente F, Halberg F. Secular or circannual immunomodulation of placebo (Pl) and melatonin (Mt) effects on murine breast cancer? Chronobiologia 1989; 16: 197.
74. Wrba H, Halberg F, Dutter A. Melatonin circadian-stage-dependently delays breast cancer development in mice injected daily for several months. In: Neuroimmunomodulation, Proc. 1st Int. Workshop on NIM, Bethesda, MD, Nov. 27-30, 1984, IWGN, Bethesda, 1985. p. 258-261.
75. Wu J, Sánchez de la Pena S, Halberg F, Cornélissen G, Wetterberg L, Halberg E, Lakatua D, Bingham C, Harvey J, Bazin H, Zheng T, Leung B, Tran B. Chronosynergistic effects of lighting schedule-shift and cefodizime on plasmacytoma growth and host survival time. (Chronoimmunomodulation by an Antimicrobial Agent, 1989, F. Carandente, F. Dammacco, F. Halberg, eds.) Chronobiologia 1988; 15: 105-128\.
76. Yunis E, Dubey D, Halberg F. Chronoimmunology--a perspective. In: Chronobiology and the Digestive System, Proceedings of a Meeting held at the University of Minnesota in September 1981, U.S. Department of Health and Human Services, NIH Publ. #84-857, May 1984. p. 73-94.

ASTRONOMY AND MEDICINE: CHRONOBIOLOGY AND FRANZ HALBERG

Philip J Regal
University of Minnesota
Ecology, Evolution, and Behavior Department
1987 Upper Buford Circle
St. Paul, Minnesota 55108

Tel. 612 343-5590 Fax 612 624-6777
Email: regal001@tc.umn.edu
http://biosci.umn.edu/~pregal/phil.html
http://biosci.umn.edu/~pregal/biosafety.html

Men sought to chart the rhythms of the heavenly bodies and they experimented with herbs to heal the body well before recorded history. Astronomy and medicine in this sense may well be the deepest roots of scientific investigation.

Men with curious minds often take an interest in both, and even search for links between the rhythms of the heavenly bodies and health. Once writing was invented, the names of Hippocrates, Galen, and Avicenna were recorded, for example.

It is fascinating to consider that the man who has made so many essential contributions to the development of Chronobiology has also in effect brought astronomy and medicine back together again, and now on a modern scientific basis.

Discovering the Internal Community of Semi-independent Rhythms

Biological rhythms are ubiquitous. Before Franz Halberg was born the world knew, of course, that there were rhythms in flower opening and leaf movements, in bee activity, in the wheel running locomotor rhythms of rodents, and obviously in our own sleep-wakefulness patterns, and these were eventually shown to be endogenous.

What Halberg first did was to reveal a new internal world of highly orchestrated multiple and semi-independent physiological rhythms. Fly a rodent or a human across the ocean, or do the equivalent by changing the light schedule in the laboratory, and monitor what is happening in and under the skin. There are many physiological rhythms, and clearly each is semi-independent because it adjusts to the new time at its own rate. Thus he developed a new insight into basic physiological organization. Moreover, he devised

model laboratory protocols comparable to electrophoresis where one can isolate different proteins because they travel in an electrical field at different rates and thus make a trail of separated dots on a gel.

These studies offered a new way of thinking about and studying physiological organization. Suppose by analogy that a traveler started hearing up the road a sound coming out of a big building and it would get louder and softer, louder and softer. The analogy here is to a rat speedily running inside a wheel in its cage and then quietly resting, running again and resting again, etc. Perhaps, scientists were hoping, there is a simple explanation for whatever mysterious physiology is driving the rat, and the naive traveler might expect a simple cause of the sounds inside the mysterious building.

But then one enters the building and starts hearing not at all some simple engine humming energetically then slowly and so on, but instead hears subtle melodies and intricate changes in tempo being played on a variety of instruments. One is in a concert hall, and has encountered their first symphony orchestra. One finds dozens of instruments playing first allegro passionate, and then moving into soft adagio, and so on. This would be an eye opener to a new and different world of sound. And similarly the concept of multiple semi-independent physiological interacting rhythms offered a new perspective and handle on physiological organization.

Fashioning the Tools for Analysis

Opening this door would in itself be the lifetime achievement that any scientist could hope for. But Franz Halberg had the vision and the creative imagination to do even more once he had opened the door to the concert hall where the rhythms that make up our physiology play their tunes.

He developed statistical methods and experimental laboratory models to begin to probe this complex new world and chart it with regard to our basic understanding of biology. Hormones cannot run a wheel, and the internal rhythms are not so easy to see. The internal music can in this sense sometimes be more like jazz than Bach or Beethoven. Yet there is chronomic structure in physiological variability just as there is structure in jazz. So new statistical methods had to be developed to pursue his vision of a true science of the time structures within organisms. Halberg developed a statistical microscope and showed how to use it as an analytical tool for characterizing physiological rhythms.

Rhythms in the Clinical World

Still -- this story is far from over. Next comes the fascinating story of how the rhythms of nature are beginning once again to join with medical science.

Professor Halberg the biologist was also Franz Halberg M.D. He very quickly grasped, and more clearly than any of the other workers on biological rhythms, that these new insights could ultimately have clinical implications and could lead the way to new ways of diagnosing disease and poor health, and indeed could lead to new ways of health enhancement and treatment. He was mapping out a long steep and difficult trail, but he forged ahead and also blazed the way for many others.

The basic premise was simple. The more physiological information we have the more likely we are to be able to diagnose and treat. By analogy the electrocardiogram gives more information than the stethoscope, and this can improve diagnosis and lead to more effective treatments.

But there were huge rocks in the trail. Mostly they were economic. But there were also philosophical rocks -- or at least if not true Philosophy they were "perspectives" at the

shadowy interface between economics and philosophy, which is where so many attitudes in life are situated.

One big rock was and is the fact that in order to map chronomes clinicians would have to increase the amount of data that they would have to collect on patients, and this would be a burden. Not to be deterred, Halberg the physician hammered away to make the rock smaller. He worked for decades to develop education programs in self-measurement and to persuade instrument manufactures to design small and convenient portable self-measurement devices. His passion had blossomed into a crusade.

The crusade was firmly based on new science that was revealing the body to be a community of many individual and yet interacting rhythms.

The data from the new scientific approach was supporting Halberg's vision of medical chronodiagnosis and chronotherapy. By monitoring rhythms such as those in blood pressure, it did prove possible to develop early warning criteria for at least some diseases. That is, when the violin section in the physiological orchestra had been playing progressively off tune, and was starting to confuse the woodwinds, which in turn was starting to confuse the brass section, this could not be heard before from outside of the concert hall, and so no one thought to step in and correct such situations.

In addition to such chronodiagnosis, Halberg organized coworkers to pioneer a scientifically disciplined chronotherapy.

Ecclesiastes 3:1-8 insists that for everything there is a season and a time for every purpose under heaven. A time to heal, and a time to break down, and a time to build up. Modern chronobiologists began discovering conditions for which there would indeed be a best time to give medications, to make organ transplants, for the malnourished to eat, and so on.

The very successes of Chronobiology with regard to the progress of basic scientific understanding, medical diagnosis, and clinical applications bring into sharp focus, however, the challenge that these findings raise for the ways in which medicine on the whole is organized and funded. Challenges in economics, tradition, policy, and "philosophy" merge here.

Mainstream medical practice has progressively committed itself to industrial pharmaceutical and instrumentation technologies. The industrial/market approach searches for simple scientific principles that can be turned into marketable products. Clinicians then broker the sales of these products and associated treatments to sick people. It is a philosophy or policy that in a great many cases works wonderfully to help many sick people.

Even more people might be helped, however, by exploiting Chronobiology selectively or fully. But fully exploiting its potential for health would require an adjustment in conventional outlook. In the most extreme scenarios, for example, the patient would no longer simply bring the body in for repairs, but would become a considerably more self-aware and involved participant. And a medicine fully educated in Chronobiology would be more open to research into new types of physiological complexities, and it would have to expand on the simple "magic bullets" approach to diagnosing and treating illness.

This was a far-sighted course to chart in the 1960s and 70s. It was difficult then for other practitioners to imagine that by the end of the millennium more people would be taking individual responsibility for their own health, or the trends in the miniaturization of technology and in rapid data processing that would emerge.

It was also a bold course to chart because in some ways it seemed superficially that while chronodiagnosis and chronotherapy might save lives, they might also make life

more complicated for physicians, rather than more simple. Understandably, not all in the medical profession welcomed something that might slow down the processing of patients, even if it was effective. Yet true to the scientific spirit Halberg and his coworkers continued to plow ahead and develop clinical sciences that exploited the potentials of Chronobiology. The science and the clinical models could be developed, even if their more widespread application would have to wait for a technologically advanced era in which the economics could be worked out, and for a public better educated in how to participate in one's own health care.

Deeper Levels of Medicine and Astronomy - New Roles for the Sun

It is relatively easy to grasp the fact that there are biological rhythms that approximate the rotation of the earth and the revolutions of the earth and moon. These astronomical cycles are familiar to everyone.

But the powerful statistical methods of Chronobiology have more recently been characterizing rhythms in the physiological concert hall that would seem strange to most people, other than astronomers.

The rhythms of light on earth are produced by the motions of the earth itself and the moon. But the sun produces many of its own rhythms, and these can be very different from our familiar rhythms of light. Dense matter and intense energies flow within the sun much like the turbulent convection cells within a caldron of bubbling soup. These moving cells generate enormous magnetic pulses that fan far out into space, and can be recorded on the surface of the earth.

Chronobiologists are finding that past and/or present influences of the magnetic pulses can be recorded as well in the physiology and behavior of humans and other organisms. Numerous publications now report rhythms that would seem utterly bizarre if astronomers were not able to show data for similar rhythms in the sun's magnetism. It is becoming clear that the sun's invisible rhythms can influence organisms on any given day, and apparently have also left their imprint, "genetic footprints," on the endogenous chronomic organization of humans and other organisms.

The more we learn, the more we learn how very much there is yet to learn. This is a good thing, for history shows that intellectual complacency can be the greatest demon that has plagued true science.

A Remarkable Career

If one scans the bibliography of Franz Halberg it will be obvious that he has been an extraordinarily busy and productive scientist, has inspired many other scientists around the world to pursue the research agendas that he has pioneered, and that he has received many honors.

But there is a problem with such a long list of accomplishments - one can get lost in it and not see the forest for the many, many admittedly interesting trees, except to see that the forest is vast and that it can be called Chronobiology.

One way to look at this forest is to try to glance away from the sea of trees and take a look at the man himself -- as a dedicated scientist, and from the perspective of the history of biology and medicine.

We have here not simply a string of important accomplishments. We have a multitude of important discoveries within the context of a truly unusual effort to take one's insights and understand the scope of their importance; then shape them in turn into a grand

scientific and humanitarian vision; and then doggedly pursue that vision on a very broad front and turn the vision into a scientific and clinical reality.

One has to think hard for historical parallels. In the basic sciences, Galileo, Newton, Darwin, perhaps Cajal did something close to this, not to slight a few others of the greats. They had insights into important and general natural phenomena, invented models of ways to gather and present data, and showed others how to pursue in a scientific manner the issues that they had worked out and outlined as a broad research agenda. In medicine Koch would be near the top of most lists, and arguably the dynamic Louis Pasteur and Paul Erlich, as the matter is framed here.

We live in an age when science is becoming evermore managed from above, and defined by managers. We should never forget that the historical progress of science has depended on individuals with insight, vision, determination, and courage and not simply on well-funded government programs and races to get "the answer" first. A tribute to Franz Halberg is well deserved for him personally, but it serves us all as well in that it serves the historical spirit of science and commemorates the integrity of that spirit.

IN MEMORIAM: ION BACIU MUTUALLY SUPPORTING NEARTRANSYEARS IN SOLAR AND TERRESTRIAL MAGNETICS, MICROBIAL AND CELL BIOLOGY, PHYSIOLOGY AND PATHOLOGY

Halberg Chronobiology Center, University of Minnesota, Minneapolis, MN, USA;
International Project on The BIOsphere and the COSmos (BIOCOS)

Ion Baciu (1921-2004) was a physiologist, investigator and teacher par excellence (1), member of the Romanian National Academy of Medical Sciences, founding member of the International Society for NeuroImmunoModulation, and with Novera Herbert Spector honorary member of the Halberg Chronobiology Center. In 1999, Spector, the father of neuroimmunomodulation, dedicated a 16-page editorial in the Romanian Journal of Physiology to Ion (2), Figure 1. In the dedication, he wrote: "More than a half-century ago, [Baciu] began to experimentally explore the influence of the central nervous system on the peripheral vascular system including the mysteriously complex actions of phagocytosis and erythropoiesis." Novera went on to describe his encounters with Ion and will honor him elsewhere. To simplify complexity and the resulting stress, when variability is as yet unassessed, Ion met with chronobiology in hematology, as it evolved from time-macroscopy to time-microscopy (3), including phagocytosis (4). For Minnesotans, it was eventually the start of an invaluable cooperation (5, 6), extending to the hard-to-define problem of wear and tear, i.e., stress/strain (7-12) and touching the transyear (13). Near- and far-transyears cnstitute a further control among many other nonphotic cycles, genetically preserved with the photic biologic day and calendaryear, as circadian and circannual rhythms. The new nonphotics as magnetoperiodisms complement the well known photoperiodisms and may enhance or dampen the latter when nearly in phase or nearly out of phase, respectively (3).

At their last personal encounter in Oradea, Ion planned with one of us (FH) on a large scale, including an institute in Oradea for chronobiology, to be headed by Germaine Cornélissen. Thereafter, we planned on an action in stroke prevention in Cluj, all awaiting implementation in Romania, delayed by Ion's failing health. One can hope that some of these plans, notably those revolving around blood pressure (BP) and heart rate (HR) monitoring in the normal range, will still come about in Ion's memory.

In this perspective, those of us who had the pleasure and privilege of cooperating with Ion on his and Monica Hriscu's actual data (5, 6), only by mail from a distance, dedicate to Ion's memory, as they already did with him as co-author (13), the finding of years related to terrestrial and solar magnetism, usually statistically significantly longer and sometimes shorter than a year. These cycles are beyond (transyear) or on the near side of (cisyear) 1 year in length. Ion wished to pursue chronobiologically qualified physiology by

deeds. Words, such as stress/strain or homeostasis, can be replaced by the actual mapping of chronomes (time structures), including rhythms, not only circadians but also others, among which transyears are a case in point. A health care and broad basic and applied transdisciplinary science all have to undergo a change from spotchecks to continuous surveillance.

In Ion's memory, we dedicate a set of new spectral components of about 1.05 years, with their respective 95% confidence intervals that do NOT overlap the calendar year, Figure 2. They were found by linear-nonlinear spectral analysis (14-16) in biology, physiology and pathology on the one hand, and in the changes of the speed of the solar wind and in the geomagnetic disturbance index aa, i.e., in helio- and geo-magnetism on the other hand. The uncertainty of the period estimate and the difference as compared to a precise 1-year length was new to physicists who discovered before us an about 1.3-year in the solar wind (17) and in the rotation speed of the solar dynamo itself (18). Similar periods, without specification of any 95% confidence intervals, had been reported (17-22), without any known attempt to scrutinize any difference between the period and the precise calendar-year.

Baciu's near-transyear is found in bacteria (23), in the eukaryotic unicell Acetabularia acetabulum (24), and in early human ontogeny in BP and HR (25-27). These biological components may mimic spectral components found in the solar dynamo by those who have used helioseismology to probe the dynamics of the sun (18) and have found that (19):

> ..rotation rates at the bottom edge of the [Sun's] convection zone, presumably providing important clues on how the solar dynamo works. The Sun does not rotate at a fixed rotation rate down there (near the base of its convection envelope). The rotation speed varies periodically, spinning fast and slow and then fast again. These alterations in rotation speed have a period of 1.3 years, or 16 months, in equatorial regions. There is a more complicated variation with a dominant 1.0-year period at higher latitudes.
>
> The periodic variations at different depths are out of phase with each other, and the contrast in speed above and below the dynamo region can change as much as 20 percent in 6 months. When the lower gas speeds up the upper gas slows down, and vice versa [...]. These pulsating relative motions between neighboring layers of electrified gas probably drive the dynamo that amplifies and generates the Sun's magnetic field.
>
> A more esoteric implication of the rotation results involves tests of Einstein's theory of gravity ...

Almost certainly, the last implication touches on diverse aspects not only of physics broader than the solar wind and geomagnetics, but also upon the relativity of time in biology. The physiological finding of concomitant different albeit close cycles in different biological variables of the same person in isolation from society may be of interest to those in physics who described about 1.3- and 1.0-yearly components on the sun itself, at different solar latitudes by helioseismology (18, 19).

The neartransyear between 1.0 and 1.2 years, with its 95% confidence interval (CI) overlapping neither of these lengths, was detected by us in the solar wind speed and in the proton density of time series downloaded from the OMNI 2 series (28). The neartransyear's period length in most cases is statistically significantly different from the precise year and from the about 1.3-year "transyear" of physics (17) or biology. Its biological counterparts with periods and CIs between 1.2- and 2.0-year length (13, 25-27) are best called fartransyears (for separation from the neartransyear reported herein).

A neartransyear was found in some but not all variables examined, not all shown in Figure 2. It represents an oscillation ascertained in a longitudinal study of the products of excretion of steroidal hormones (29) and in a hybrid (linked cross-sectional) investigation of human BP and HR in health at birth (30). It is thus found early in human extrauterine ontogeny as a feature of Ion's beloved physiology. Human pathology, such as suicide (31) and autism (32), also exhibits a near-transyear. In autism there are three different signatures of magnetoperiodism, the other two being a signature of the about 21-year Hale cycle of sunspot bipolarity and of the half-year of geomagnetic activity (33).

The finding of the same components in bacteria and in a eukaryotic unicell may be a hint that a qualified concept in spatial morphology, that ontogeny recapitulates phylogeny, does have a counterpart in the physiological structure of life early in phylogeny as well as early in human extrauterine ontogeny. Features of chronomics far beyond mere transyears complement genomics in biology, and complement the study of the solar wind. Bacteria and *Acetabularia* document as "eyewitnesses", the similar dynamics of the sun and of its wind as the latter blew for the past billion or more years, an extrapolation based on independent physical evidence as well (34). Eventually, Figure 2 may be considered for any update of Ion's classical Romanian textbook of physiology (1)?

The foregoing is a summary of a more extensive original prepared for the Leibniz Society (the former DDR Academy of Science) in Berlin (33).
For chronobiology and chronomics, Franz Halberg, Germaine Cornélissen, George Katinas, Mary Sampson, Othild Schwartzkopff and the members of the BIOCOS project
For neuroimmunomodulation, Novera Herbert Spector
For microbiology, Piero Faraone
For Ion's family, Sanda Tomescu
For Ion's academic family, Monica Hriscu

Farewell Ion, farewell stress-strain
Your preoccupation with science remains everybody's gain
From Cluj you reached out to the world
Your message in NeuroImmunoModulation, NIM, was heard
But whatever one measures, NIM or otherwise
Needs controls, an indispensable price:
In phagocytosis you found a change on the day's scale
Circadians for many became a Holy Grail
But we must explore also other variation
Changes with the year are another research station
There we found a Neartransyear now dedicated to you
As a friend in everyday physiology so true

"Din codru rupi o ramurea,
Ce-i pasa codrului de ea!
Ce-i pasa unui univers intreg
De-al meu amurg."
BUT NOT FOR ION BACIU

In Romania's scientific forrest, one tree
Ion Baciu's transyear one can not fail to see
The world may not care about you and me
But it needs the transdisciplinary control: periodicity

Franz Halberg

The help of Crina Turtoi, Scientist, Institute of Agrarian Economics of the Romanian Academy of Sciences, who in many ways, including the locating of a poem by George Cosbuc here cited in memoriam, is greatly appreciated. Our gratitude also extends to Rachel Howe, Associate Scientist, National Optical Astronomy Observatories, Tucson, Arizona, for providing the demonstration of 1.3- and 1.0-year components on the solar dynamo and data for a follow-up.

REFERENCES

1. Baciu I. Fiziologie. Bucharest: Editura Didactica si Pedagogica; 1977. 874 pp.
2. Spector NH. The NIM revolution. Rom J Physiol 1999; 36: 127-143.
3. Halberg Franz, Cornélissen G, Katinas G, Syutkina EV, Sothern RB, Zaslavskaya R, Halberg Francine, Watanabe Y, Schwartzkopff O, Otsuka K, Tarquini R, Perfetto F, Siegelova J. Transdisciplinary unifying implications of circadian findings in the 1950s. J Circadian Rhythms 2003; 1: 2. 61 pp.
www. JCircadianRhythms.com/content/pdf/1740-3391/1/2.pdf
4. Szabo I, Kovats TG, Halberg F. Circadian rhythm in murine reticuloendothelial function. Chronobiologia 1978; 5: 137-143.
5. Baciu I, Cornélissen G, Olteanu A, Halberg F. Chrono-meta-analysis of circadian phagocytosis rhythms in blood of guinea pigs on two different lighting regimens. Chronobiologia 1994; 21: 307-310.
6. Hriscu M, Cornélissen G, Saulea G, Ostriceanu S, Baciu I, Halberg F. Alcohol and melatonin influence circadian rhythmic phagocytic activity and adherence of neutrophils in rats. Read by Title, Abstract 4, Proc 4th International Symposium, Workshop on Chronoastrobiology and Chronotherapy, Kamo T, editor. Yokohama: Division of Neurology, Department of Neurology, Yokohama City Seibu Hospital, St. Marianna University School of Medicine; 8 Nov 2003. p. 30-35.
7. Osler W, editor. Modern Medicine: Its Theory and Practice, Vol. 4: Diseases of the circulatory system -- diseases of the blood -- diseases of the spleen, thymus and lymph-glands. Philadelphia: Lea and Febiger; 1908. p. 430-443.
8. Selye H. The general adaptation syndrome and the diseases of adaptation. J Clin Endocr 1946; 6: 117-230.
9. Selye H. The Stress of Life. New York: McGraw-Hill, 1951.
10. Selye H. Stress without Distress. Philadelphia: Lippincott, 1974.

11. Spector NH, Dolina S, Cornélissen G, Halberg F, Markovic BM, Jankovic BD. Neuroimmunomodulation: neuroimmune interactions with the environment. In: Fregly MJ, Blatteis CM, editors. Handbook of Physiology, Section 4: Environmental Physiology. New York: American Physiological Society/Oxford University Press; 1995. p. 1537-1550.
12. Halberg F, Cornélissen G, Spector NH, Sonkowsky RP, Otsuka K, Baciu I, Hriscu M, Schwartzkopff O, Bakken EE. Stress/strain/life revisited. Quantification by blood pressure chronomics: benetensive, transtensive or maletensive chrono-vasculo-neuro-immuno-modulation. Biomed Pharmacother 2003; 57 (Suppl 1): 136s-163s.
13. Halberg F, Cornélissen G, Stoynev A, Ikonomov O, Katinas G, Sampson M, Wang ZR, Wan CM, Singh RB, Otsuka K, Sothern RB, Sothern SB, Sothern MI, Syutkina EV, Masalov A, Perfetto F, Tarquini R, Maggioni C, Kumagai Y, Siegelova J, Fiser B, Homolka P, Dusek J, Uezono K, Watanabe Y, Wu JY, Sonkowsky R, Schwartzkopff O, Hellbrügge T, Spector NH, Baciu I, Hriscu M, Bakken E. Season's Appreciations 2002 and 2003. Imaging in time: The transyear (longer-than-the-calendar year) and the half-year. Neuroendocrinol Lett 2003; 24: 421-440.
14. Marquardt DW. An algorithm for least-squares estimation of nonlinear parameters. J Soc Indust Appl Math 1963; 11: 431-441.
15. Halberg F. Chronobiology: methodological problems. Acta med rom 1980; 18: 399-440.
16. Cornélissen G, Halberg F. Chronomedicine. In: Armitage P, Colton T. (editors-in-chief). Encyclopedia of Biostatistics, v. 1. Chichester, UK: John Wiley & Sons Ltd., 1998: 642-649.
17. Richardson JD, Paularena KI, Belcher JW, Lazarus AJ. Solar wind oscillations with a 1.3-year period. Geophys Res Lett 1994; 21: 1559-1560.
18. Howe R, Christensen-Dalsgaard J, Hill F, Komm RW, Larsen RM, Schou J, Thompson MJ, Toomre J. Dynamic variations at the base of the solar convection zone. Science 2000; 287 (5462): 2456-2460.
19. Lang KR, ed. The Cambridge Encyclopedia of the Sun. Cambridge: Cambridge University Press; 2001.
20. Shapiro R. Interpretation of the subsidiary peaks at periods near 27 days in power spectra of geomagnetic disturbance indices. J Geophys Res 1967; 72: 4945-4949.
21. Silverman SM, Shapiro R. Power spectral analysis of auroral occurrence frequency. J Geophys Res 1983; 88 (A8): 6310-6316.
22. Mursula K, Zieger B. The 1.3-year variation in solar wind speed and geomagnetic activity. Adv Space Res 2000; 25: 1939-1942.
23. Faraone P, Cornelissen G, Konradov A, Vladimirskii B, Chibisov S, Katinas G, Halberg F. A transyear in air bacteria and staphylococci. Abstract, Biological effects of solar activity, Pushchino, Russia, April 6-9, 2004, unpaginated.
24. Halberg F, Cornélissen G, Faraone P, Schwartzkopff O, Regal P, Holley DC, Otsuka K. A transdisciplinary near-transyear in Acetabularia validates its counterpart in the solar wind and geomagnetics. Abstract, Proc 5th International Symposium, Workshop on Chronoastrobiology and Chronotherapy, Tokyo, 2004, in press.
25. Halberg F, Cornélissen G, Schack B, Wendt HW, Minne H, Sothern RB, Watanabe Y, Katinas G, Otsuka K, Bakken EE. Blood pressure self-surveillance for health also reflects 1.3-year Richardson solar wind variation: spin-off from chronomics. Biomed Pharmacother 2003; 57 (Suppl 1): 58s-76s.
26. Cornélissen G, Masalov A, Halberg F, Richardson JD, Katinas GS, Sothern RB, Watanabe Y, Syutkina EV, Wendt HW, Bakken EE, Romanov Y. Multiple resonances

among time structures, chronomes, around and in us. Is an about 1.3-year periodicity in solar wind built into the human cardiovascular chronome? Human Physiology 2004; 30 (2): 86-92.
27. Halberg F, Cornélissen G, Schack B. Self-experimentation on chronomes, time structures; chronomics for health surveillance and science: also transdisciplinary civic duty? Behavioral and Brain Sciences, http://www.bbsonline.org/Preprints/Roberts/Commentators/Halberg.html
28. King J, Papitashvili N. OMNI 2 documentation. http://nssdc.gsfc.nasa.gov/omniweb/ow.html "The OMNIWeb interface provides access to the multi-source OMNI 2 data set, to the individual data sets contributing to OMNI 2 and to detailed documentation about OMNI 2."
29. Cornélissen G, Halberg F, Hamburger C, Perfetto F, Tarquini R, Ungar F, Otsuka K, Chibisov S, Breus T, Schwartzkopff O. Chronomics: multiple distinct yearly cycles in urinary steroid metabolite excretion. Abstract, 2nd International Symposium, Problems of Rhythms in Natural Sciences, Moscow, March 1-3, 2004. Moscow: Russian People's Friendship University; 2004. p. 17-18.
30. Cornélissen G, Syutkina EV, Johnson D, Otsuka K, Halberg F. Near-transyears and transyears in geomagnetics and biology. Abstract, Proc 5th International Symposium, Workshop on Chronoastrobiology and Chronotherapy, Tokyo, 2004, in press.
31. Cornélissen G, Palermo J, Carandente F, Halberg F. Chronomics: chronome of suicides in Minnesota (1968-2002). Abstract, MEFA, Brno, Czech Republic, Nov. 2-5, 2004, in press.
32. Halberg F, Cornélissen G, Panksepp J, Schwartzkopff O. Near-transyears in geophysics and autism. Abstract, MEFA, Brno, Czech Republic, Nov. 2-5, 2004, in press.
33. Halberg F, Cornélissen G, Schwartzkopff O, Syutkina EV, Katinas GS, Engebretson M, Faraone P, Masalov AV, Bingham C, Schweiger H-G. Sich gegenseitig stuetzende Nahtransjahre im Sonnenwind, Geomagnetik und Biologie: drei Magnetozyklizitäten im Autismus. In preparation.
34. Steiger R v. History of the solar wind. In: Schröder W (editor). Arbeitskreis Geschichte der Geophysik und Kosmische Physik. Meteorological and Geophysical Field Dynamics (A book to commemorate the centenary of the birth of Hans Ertel). Bremen: Wilfried Schröder/Science Edition/Arbk Geschichte Geophysik: 2004. p. 75-89.

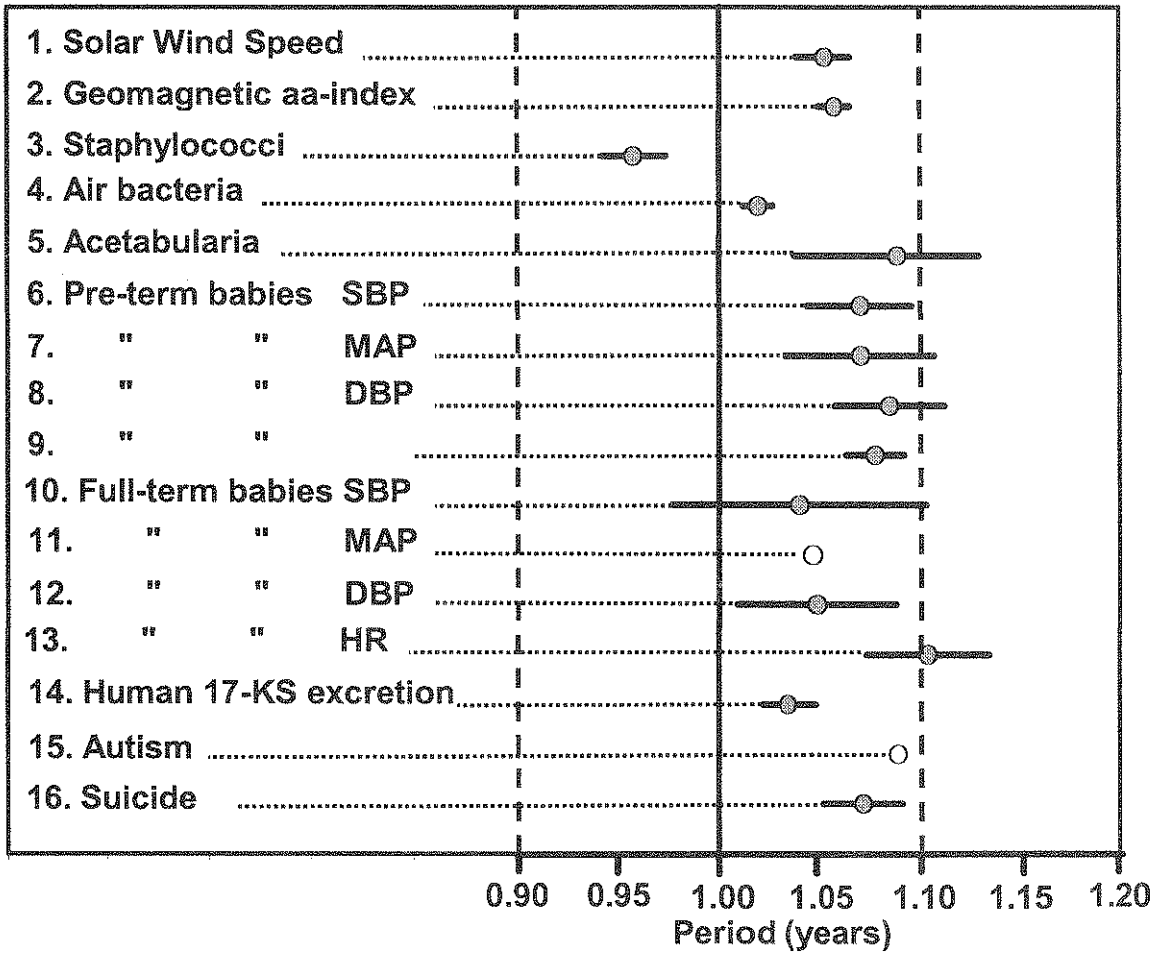
Legends

Figure 1. Ion Baciu, in his forties and in his fifties.

Figure 2. Transyears with their uncertainties. It is anticipated, however, that the dynamics of the sun are largely independent of the movements of the earth around the sun, and the solar dynamo apparently has a distinctly different period, demonstrable as longer than a year, as the major component in the spectrum. With this finding, on the one hand in physical variables like changes in the speed of the solar wind and in proton density of the solar wind (the latter not shown), and on the other hand in biological variables being mutually reinforcing, the reality of their occurrence and the rule that the periods that have characterized the solar system, whether of photic or non-photic origin, have been genetically preserved and constitute a structure in time resolvable by spectral analysis.

Figure 1

CHRONOMICS:
NEARTRANSYEARS AND A NEARCISYEAR AROUND (1, 2) AND IN (3 - 16) ORGANISMS



1 = data from OMNI 2; 2 = first 131 years; 3, 4 = sectoring, i.e., probable mutations and/or other genetic changes; 5 = Circadian acrophase of oxygen production during 14 years; 6 - 13: S = systolic, MA = mean arterial, D = diastolic, BP = blood pressure, HR = heart rate; 14 = daily excretion for 15 years of urinary steroidal metabolites (17-ketosteroids, CH); 15 = incidence in California; 16 = incidence in Minnesota. Circle: period, point estimation: gray or white if 95% confidence interval of amplitude does not overlap ($P<0.05$) or overlaps ($P>0.05$) zero, respectively; horizontal line through circle: conservative 95% confidence interval.

Figure 2

CHRONOBIOLOGY OF ACUTE AND CHRONIC DISEASE EVENTS

Thomas KENNER, Klaudia TERTSCHNIG
Karl-Franzens-Universität Graz
Department of Physiology
Graz, Austria
Thomas.Kenner@kfunigraz.ac.at

INTRODUCTION

Franz Halberg (1) was the first to use the term chronome for the description of the entirety of biological effects related to internal biological clocks and to the interaction with external Zeitgeber. Thus, the chronome is equivalent to the individual biological time structure of an organism.

Greek words are used in science and medicine to underline facts of particular importance. The word chronos for time is included in "chronobiology", which is the complete field of time-related phenomena in biology and medicine. Chronobiology describes normal healthy, and pathological mechanisms of biological systems, all of which are related with or interact with the chronome of the system.

Since there is nothing existing outside a time frame, chronobiology has to include any aspect of time in biological systems. In essence, we can state that biology is identical with chronobiology.

The word chronome indicates the existence of a time structure of an organism and implies a relation to the genome.

The spectrum of biological rhythms extends from milliseconds to lifetime and may even include the life cycle of societies. Concerning the basic concept of chronomics, the biological control of biological rhythms is based on hierarchical sets of control systems with time delay and negative feedback. Disturbances from internal and external sources can interfere with biological rhythms.

Acute and chronic

In the description of diseases the word chronos is included in chronification and in the corresponding adjective chronic. Chronification describes a process which extends a pathological condition or event over a prolonged period. In contrast to chronic, acute is used to describe a pathological process, which starts suddenly and ends after a short period either with death or with recovery or is extended by chronification. An exact definition of a limit between acute and chronic is impossible or is quite vague.

Any disease, be it acute or chronic or be it a sudden and unexpected event (like e.g. sudden infant death, or as an accident) has a preceding history - a "strategy of disease". In infections this history includes behavioural risk, inborn strength of defence mechanisms and incubation time.

Example 1

Any state or condition of a complex system at a certain time depends on its history. The following example demonstrates how a specific and apparently singular biological event - the contraction of the heart - is markedly influenced by the preceding history of this event. We could illustrate this phenomenon by a study on the time sequence of arrhythmic arterial pressure pulses by methods of time series analysis. It can be shown by time series analysis that each consecutive blood pressure amplitude depends markedly at least on properties of 5 or even more preceding beats (2). The heart seems to have a short-time memory on what had happened a few contractions ago. The cellular process on which this kind of memory process is based, is the uptake and release of calcium by the myocardial cells. A phenomenon which was described by Bowditch (3) quite some time before the discovery of Frank and Starling of the "law of the heart", can be found in physiology text books under the key words "stair case phenomenon" or more recently "post extrasystolic potentiation".

Example 2

This example demonstrates the cooperation of influences and factors which, as in the first example, act in a time dependent sequence. In clinical medicine, the present condition of a system or of an organism at a certain moment of time depends on the whole life history. In discussing a disease the influencing factors - they may be called protective factors or risk factors, respectively - can be subdivided into three categories: 1. Factors which have something to do with age or with the degree of previous development. 2. Factors which increase the individual vulnerability and/or deteriorate internal conditions. 3. The outbreak of disease may be triggered by a critical occasion or by an exogenous event.

An example of this "triple hypothesis" was proposed in connection with the explanation of risk for sudden infant death (SIDS) as summarized by Kurz, Kenner and Poets (4). In the case of SIDS the three groups of risk factors can be summarized in short: critical child, critical time, critical trigger.

In contrast to the first example the preceding history prepares an acute, an unexpected and sudden, extremely dangerous situation.

The following more complex sequence of events, the "strategy of disease" which may lead to a sudden and unexpected apparent life threatening event (ALTE) or to the sudden and unexpected death of an infant (SIDS) is hypothesized: 1. In the time of the second and third month of life the development of the brain of an infant has reached a state where a "major transformation" (5) takes place. 2. As a consequence the individuals vulnerability is increased. Furthermore, a majority of babies whose death was diagnosed as SIDS was found in prone position. There is recently extensive information available concerning sleeping position, temperature, clothing, condition of baby's bed, effect of passive smoking etc. In Styria our SIDS-prevention- and information-project had very a favorable success (6). 3. The most probable sequence of pathophysiologic events which may lead to death can be summarized as follows: baby sleeps in prone position > REM sleep > reduced muscle tone > head may be in mid position > because of muscle relaxation and soft pillow support the baby is not able to turn his head aside > arousal mechanism is unable to overcome the situation > apnea and progressive bradycardia > which finally may lead to cardiac arrest.

The sequential strategy of disease

Each physician certainly knows such sequences of events which acutely or chronically progressing may modulate a disease process. In earlier time physicians were then hoping for the so called crisis which in the positive case then may lead to recovery.

Interestingly enough there is recently much discussion about a mathematical description which appears to be a model a disease sequence (7). Expressed in words the model states that the event Nr. N+1 is a function of event Nr. N. The result, of course depends on the kind and details of the function. But even a rough consideration brings some interesting consequences: As already included in the time series model of the first example, this second model implies again, that chronobiological phenomena may be generated by a sequence of time periods. Furthermore, the type of equation of the second model may - depending on the exact formulation and on the chosen parameters - lead to following solutions: monotonous growth, different periodic oscillations and finally, description of a chaotic sequence (7).

It seems that chronification leads to a pattern of disease, which more closely resembles chaos.

Example 3

One of the most frequent chronic complains of persons in higher age in western society is pain, and particularly back pain (8). We can try to adjust the three categories of "the strategy of disease" - as it was mentioned with the second example:

1. Factors, which have something to do with age or with the degree of previous development. In aging people this may most probably be spondlyarthrosis and some consequences of osteoporosis. 2. Factors, which increase the individual vulnerability and/or deteriorate internal conditions. In spite of extensive information the following mistakes are made: no attempt to reduce overweight, no gymnastics. 3. The outbreak of disease may be triggered by a critical occasion or by an exogenous event. Then, the following pathophysiological effects which inhibit healing, take place: pain as such tends to be perpetuated on a neural level. This effect is aggravated through psychological factors and particularly, emotions. Life history plays an extremely important role. Chronification is essentially based on the effects of information storage and memory.

Conclusions

The chronobiological implications of the following daily problems of medicine are: 1. The development of an acute event, and 2. chronification.

An acute attack of a disease event - e.g. ALTE, Apparent Life Threatening Event - or an accident, or sudden death, can only be understood if as much as possible is known about the preceding "strategy of disease".

The history of influences and their interaction may through a slightly different "strategy of disease" lead to chronification. The term chronification describes the time dependent process, which from a single initial impact leads to a continuing deviation from normal, which continuously is stored by some kind of memory. Any disturbed system needs a certain characteristic time to reestablish its normal condition. Any process, which inhibits the correction of the variable, may lead to chronification. Chronification may be generated externally by repetition or continuation of a disturbance, which may be due to a disturbance of the characteristic properties of the chronome.

Using a very simplified description of a mathematical model which explains the step by step development of a pathological as well as a physiological process it becomes clear that

in each of the possible results the chronome of the organism is changed. The possible resulting time patterns, which are produced by the disease process are: monotonous change, new periodicity and chaos. The latter most probably is valid to describe chronification. It can be hypothesized that this time course, which is generated by the disease process interferes with the existing internal chronome. The result may be on one hand generation of complex interference pattern, on the other hand synchronization of both patterns. Such effects may explain sudden deterioration or even break down in the course of a chronic disease process.

ABSTRACT

Chronic diseases and the process of chronification belong - as chronobiology - to the words with the Greek component "chronos" - time. Of equal importance is the consideration of acute diseases which in spite of lacking chronos nevertheless play a role in terms of the time course of the disease. With 3 examples the idea of the "sequential strategy of disease" is explained. It is essential that this strategy has to include history and, therefore, depends on memory. All diseases have a characteristic time course and thus may force a restructuring of the chronome. A simple verbal description of a model-equation explains the possibility that a basic process of a sequence of time periods generates the time dependent characteristics of any disease.

LITERATURE

1. Cornélissen G, Halberg F: Introduction to Chronobiology. University of Minnesota, 1994
2. Hardman S.M.C., Pfeiffer K.P, Kenner T., Noble M.I.M., Seed W.A.:
   Analysis of left ventricular contractile behaviour during atrial fibrillation
   Basic Research Cardiol. 89: 438 - 455 (1994)
3. Bowditch HP: Über die Eigenthümlichkeiten der Reizbarkeit, welche die Muskelfasern des Herzens zeigen. Berichte aus der königl. Sächs. Ges. d. Wissenschaften 23: 652-89 (1871)
4. Kurz R, Kenner T, Poets C (Hrsg.): Der Plötzliche Säuglingstod.
   Springer-Verlag, Wien, New York, 2000
5. Prechtl HFR:
   General movement assessment as a method of developmental neurology: new paradigms and their consequences.
   Dev Med Child Neurol. 43 836-842. (2001)
6. Styrian Sudden Infant Death Study Group.
   Classification of sudden infant death (SID) cases in a multidisciplinary setting. Ten years experience in Styria (Austria). Wien Klin Wochenschr. 115: 887-93 (2003)
7. Bassingthwaighte JB, Lieboivitch LS, West BJ: Fractal Physiology. (pp: 120). Oxford University Press, NY, Oxford 1994
8. Gralow I: Psychosoziale Risikofaktoren in der Chronifizierung von Rückenschmerzen. Schmerz 14: 194 - 110 (2000)

CONTROL OF TEMPERATURE IN MEN IN SENEGAL

Martineaud J.P. and Cisse F.*, Guéye L., Samb A., Seck D

Faculty of Medicine, St.Louis-Lariboisiere Hospital, Paris, France and *Faculty of Medicine, Dakar, Senegal

INTRODUCTION

Mammals and birds are characterized by a very stable average body temperature in physiological conditions with limited and regular fluctuations over 24 hours, because normal tissue metabolism requires a precise and constant thermic environment. Furthermore it was well demonstrated that fluctuations in central temperature are periodic and mainly generated by periodic variations in heat production and heat loss, depending on alternation of day and night and concomitant changes in environmental temperatures, professional work, food intake, participation in social life. All these influences are closely interconnected and determine regularly cyclic increases and decreases in core temperature. Nevertheless if we may observe a circadian rhythm in body temperature, the period amplitude of this phenomenon is not constant. Thus some physiologists have observed in the hottest areas of Asia or Africa that big mammals could have central diurnal temperatures higher in summer than in winter. In man, it was observed that the amplitude of the central temperature was not absolutely constant. Then in order to know how some external factors could influence the maximal and minimal levels of body temperature, a series of measurements was carried out with exposure to temperate and hot temperatures with and without food intake in subjects naturally acclimatized to hot climates.

MATERIAL AND METHODS

All experiments were carried out in the intertropical area of western Africa : Senegal, Mali, Togo., between 10 and 13° north latitude. The aim of this study was to determine the influence of increased ambient temperatures on the central (or core) temperature in man and its modifications with normal alimentation or during fasting. We carried out our experiments during winter (moderate climate) and summer (hot season).

The first and second series of measurements on circadian rhythm of central temperature were carried out in Kedougou (western Senegal) during the months of February (moderate ambient temperature) and June (hot period) in normal dietetic conditions. 50 healthy young men (19-26 years old) participated in two trials, for

comparison of responses of their circadian cycle in both situations in order to determine the exact influence of two external stresses.

The third and fourth series of measurements were conducted in Dakar, sea-sided capital of Senegal during the month of March to establish a possible influence of feeding on central temperature. 22 healthy young men (20-25 years) were tested successively with normal alimentation and during a 24 h fasting, when the environmental temperature was moderately elevated. The last series of measurements was performed in various sites in Senegal (Dakar, Podor, Ourosogui, Kedougou), Mali (Bamako), Togo (Lome) to determine body temperatures in very different temperatures (20 to 47 °C), either in the morning or in the afternoon. 700 subjects, all males (18-65 years) participated in this study.

RESULTS

In the first and second series of rectal temperatures determination, we observed at first normal variations of core temperature , in winter as well as in summer, with the highest values in the afternoon and the lowest at night. Furthermore these minimal values were not statistically different according to the season : 36.40 in winter and 36.30 in summer. Comparisons between values of the other components of the circadian cycle showed highly statistically significant differences : in summer the highest ambient temperature was associated with an increase in MESOR - approximately 24 h mean value) by 0.26 (MESOR in summer, 37.09, MESOR in winter, 36.83) and an increase in the circadian temperature amplitude (double amplitude corresponds to the difference between the highest and the lowest values) by 0.25 (amplitude in summer 0.50°C, in winter 0.25); there was a difference of 60 min in the delay for the circadian acrophase (interval between midnight and the highest day time value, in winter 4.30 PM, in summer 5.30 PM).

For the third and fourth experiments, comparison of two series of determinations showed statistically significant differences for the circadian components of central temperatures. Fasting was associated with a decrease in MESOR by 0.19°C (MESOR during fasting, 36.65°C, in normal dietetic conditions, 36.84), a decrease in the circadian temperature amplitude of 0.09°C (in fasting, 0.40°C, in normal conditions, 0.49) and a delay in acrophase, 50 min (in fasting, 4.50 PM, in normal conditions, 4 PM). The lowest values of core temperatures observed between 3 and 6 AM were not different, however, 36.35°C during fasting, 36.30 with normal dietetics.

In the last series of measurements, we could establish two statistically significant correlations between central (Tc) and ambient (Ta) temperatures using a number of measurements in various places. First in the morning, at Ta = 20°C, Tc was 37.25°C and at Ta = 35°C, Tc was 37.40°C, and between these two points, there was a statistically significant linear relationship. Then with ambient temperatures higher than 35-36°C, and up to 46°C, there was no more increase in body temperatures. The constant value for central temperature independent of environmental temperatures was 37.55°C.

DISCUSSION

Internal temperature is maintained constant in human beings in spite of great variations in environmental conditions: indeed thermoregulation is characteristic of the human life, but mammals and birds have the same capacity. In man, there are physiological but limited modifications of the body temperature, regular over 24 h (circadian cycle), periodic with the menstrual cycle in women, circumstantial with feeding

and physical activities. On the other hand, it is known that in desert and arid environments mammals can survive developing adaptations allowing them to withstand high increases in internal temperature during very hot days. For example, the body temperature of camels may vary considerably: in the absence of heat stress, the daily fluctuations are about 2°C, but in extreme conditions the variations may reach 6°C. This is of significance in the water economy which is a real problem in desert environment. The prevailing external temperature fall during the night makes easier and cheaper the heat dissipation. Consequently desert animal species have no unusual ability in thermoregulation but tolerate increased body temperatures. Various authors reported that the body temperatures of residentially acclimatized men in humid heat were higher than those of men living in temperate climates. Then the question is : does the human organism possess any physiological mechanism for coping with the highest ambient temperatures and increased body temperature. Our first series of measurements (1 and 2) demonstrate that the 24 h cycle is still present at temperatures as hot as 45°C and that thermoregulation is still efficient. There is a clear adaptation of the circadian cycle during the hottest hours of the day in summer while the internal temperature increases more than usually and the organism waits for the night when energy and water economy is as cheap as possible to dissipate heat.

Among the factors increasing the metabolic rate and consequently the internal temperature, food intake, especially proteins is an important one. Then the second question is: what is the origin of the increased body temperatures during exposure to heat stress or only environmental temperature or with participation of a nutritional factor? The second series of experiments (3 and 4) demonstrated that a nutritional element is present in hyperthermia. Indeed the thermoregulatory system doesn't correct entirely the protein-induced thermogenesis. But with our experiments it was not possible to ensure that it's the only factor responsible.

The third series of measurements in usual conditions at very varying ambient temperatures (5 and 6) allowed us to observe a linear relationship with body temperatures in the morning as well as in the afternoon, i.e in fasting situation and after food intake. Of course the slopes of two regression lines were slight but significant correlations were present, showing unquestionably that environmental temperature modifies the responses of the human thermoregulatory system. Furthermore numerous measurements above 33°C of ambient temperature disclosed an upper limit for the relationship, approximately 37.5°C for the body temperature. This corresponds to 35-37°C for ambient temperatures and for higher values there was not supplementary increase in core temperature. So above this level there was a more efficient reaction of the thermoregulatory system in order to maintain the body temperature in good conditions for tissue metabolism.

CONCLUSIONS

The studies carried out in the subtropical zone during temperate (winter) and hot (summer) seasons demonstrated that the circadian cycle of body temperature was present in all circumstances, but with increase in his amplitude during the hottest season, by increase in the diurnal values, revealing a physiological adaptation of the thermoregulatory system to the environment. This increase was at least partially due to the food intake, particularly proteins inducing a specific thermogenesis (4, 5, 6, 7). Furthermore a relationship was established between central and ambient temperatures with an upper limit. This limit was approximately 37.5°C for the central temperature,

corresponding to 35-37°C for environmental temperatures (8, 9, 10, 11). For higher values human organism becomes again a strict homeotherm, showing the limits of physiological adaptations to the thermic stress.

REFERENCES

1. Cisse F, Martineaud JP. Circadian cycles of central temperature in hot climate in man. Arch Internat Physiol Bioch Biophys 1991;99:155-159.
2. Cisse F, Ndoye R, Martineaud JP. La température centrale et son rythme circadien chez l'homme acclimaté a la zone intertropicale. Dakar Médical. 1992; 37: 171-177.
3. Martineaud JP, Cisse F, Samb A. Circadian variability of temperature in fasting subjects. Scripta Medica 2000; 73:15-24.
4. Astrand PO, Rodahl K. Physiologie de l'éxercise musculaire. Paris ; Masson,1992.
5. Cisse F, Martineaud JP, Ndoye R, Gueye L. Retentissement du jeune du Ramadan sur l'exercice musculaire submaximal. Méd du sport 1993 ; 67:76-80.
6. Dohm GL, Beeker RT, Israel RG, Tapscott EB. Metabolic responses to exercise after fasting. J Appl Physiol 1986; 61: 1363-8.
7. Elati J, Kallal Z, Beji C, Danguir J. Comportement alimentaire durant le Ramadan. Répercussions sur les parametresd métaboliques, énergétiques et hormonaux. lerCong Internat "Santé et Ramadan" 1994;159-73.
8. Zhang JQ, Thomas TR, Ball SD. Effect of exercise timing on postprandial lipemia and HDL cholesterol subfractions. J Appl Physiol 1998 ; 85 : 1516-22.
9. Rieu M. Bioénergétique de l'éxercise musculaire et de l'entraînement physique. Paris: Presses Universitaires, 1988.
10. Pequignot JM, Peyrin L, Peres G. Catecholamine fuel interrelationships during exercise in fasting man. J Appl Physiol 1980 ; 48 : 109 - 13.
11. Nielsen M. Thermoregulation in rest and exercise. Acta Physiol Scand 1969 ; suppl 323 : 1-74.

CIRCADIAN RHYTHM OF MELATONIN IN RAT DUODENUM

Michal Zeman[1], Germaine Cornélissen[2], Katarina Balazoval, Rita Jozsa[3], Andras Olah[3], György Nagy[4], Valer Csernus[3], Jozsef Kaszaki[5], Weihong Pan[6], George Bubenik[7], Franz Halberg[2]

[1]Comenius University, Bratislava, Slovakia; [2]University of Minnesota, Minneapolis, MN, USA; [3]University Pecs, Hungary; [4]Semmelweis University of Medicine, Budapest, Hungary; [5]University of Szeged, Hungary; [6]Pennington Biomedical Research Center, Baton Rouge, LA, USA; [7]University of Guelph, Guelph, Ontario, Canada
Support: ETT 82/2003 (RJ), VEGA1/1294/04 (MZ)

Background

Circulating melatonin is known to be circadian periodic in many species, peaking during the night (dark span) in both diurnally- and nocturnally-active mammals.

Aim

Whereas the circadian rhythm in circulating melatonin is well known, this study aimed at assessing whether it could also be determined in the duodenum of rats.

Materials and methods

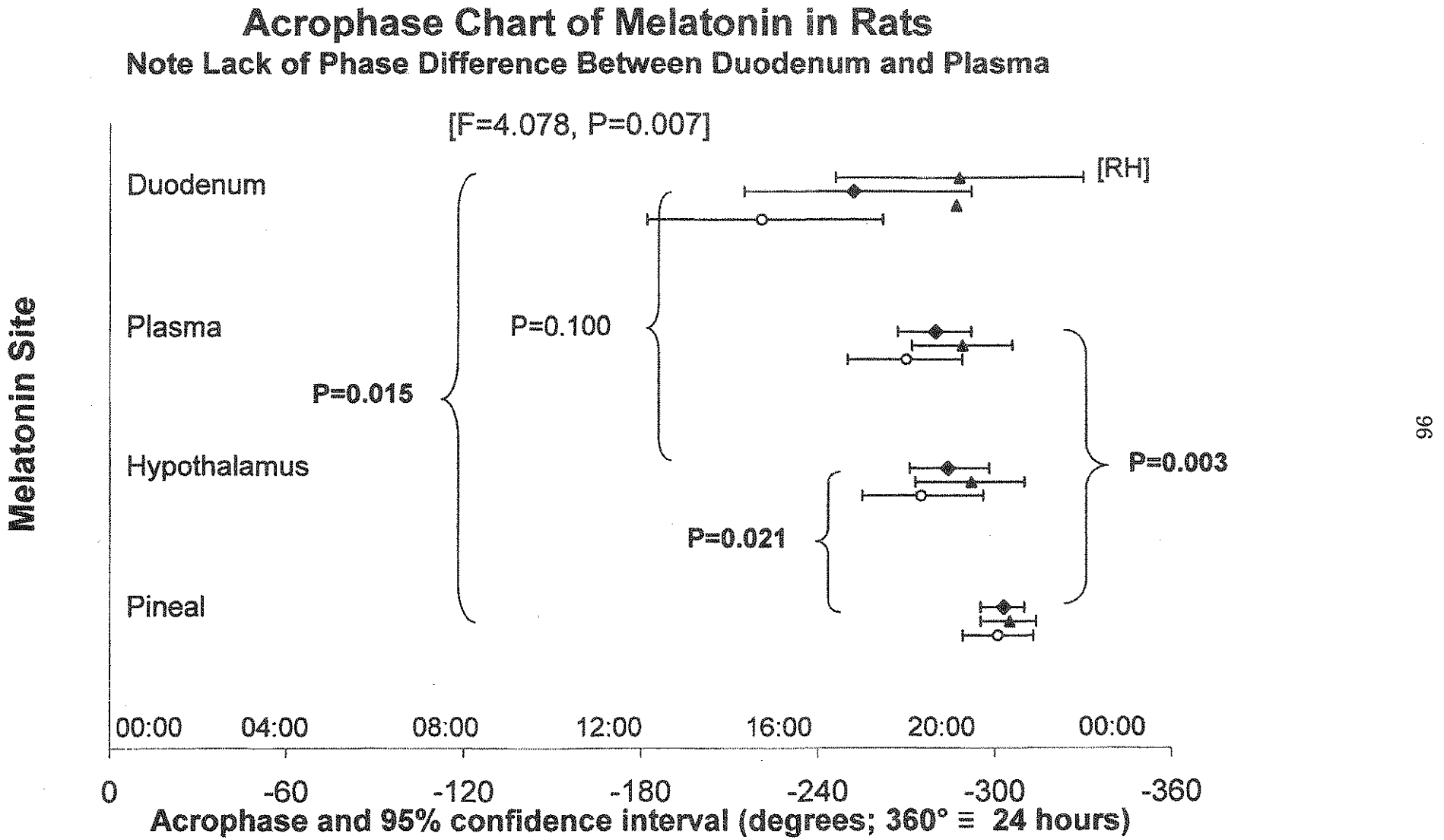
As part of a circadian-circaseptan study, a total of 102 rats (52 males and 50 females) were subdivided into two rooms kept on opposite LD12:12 regimens, to be sampled only during working hours at 4-hour intervals (6 circadian stages) for 7 days. Melatonin was determined by RIA in plasma and in pineal, hypothalamic and duodenum tissue. Duodenum melatonin could be determined on only 31 samples. The data were analyzed by cosinor and by one-way analysis of variance for any circadian variation.

Results

A circadian-stage dependent effect is of borderline statistical significance by one-way ANOVA on the original data ($F=2.279$, $P=0.078$). After log10-transformation to normalize the distribution, timepoint means are found to differ with statistical significance ($F=3.924$, $P=0.009$). A circadian rhythm is demonstrated by single cosinor overall (PR=26%, $P=0.016$), peaking about 16.8 hours after light onset. The circadian variation is similar for male (PR=34%, $P=0.057$) and female (PR=48%, $P=0.026$) rats, with acrophases occurring about 19.1 and 14.7 hours after light onset, respectively.

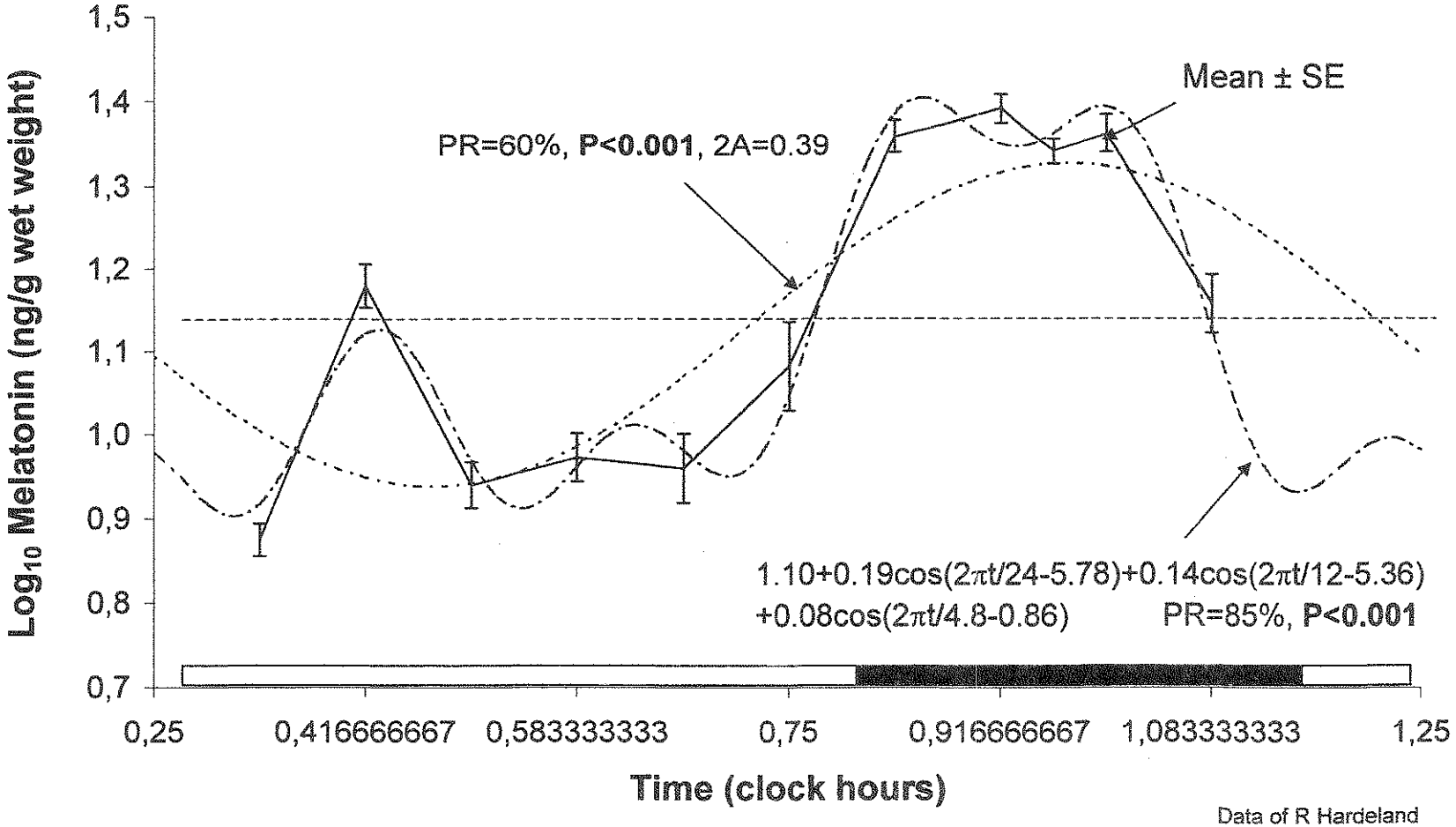
Discussion and Conclusion

This study shows the practicability of using staggered lighting regimens to map melatonin rhythms in the laboratory with sampling restricted to the usual working hours. Even on a limited sample, a circadian component could be detected for melatonin in the duodenum of rats. Most important, the study demonstrates a lead in phase of the melatonin rhythm in the gut and plasma vs. that in the pineal, awaiting replication.



Key: diamonds: All animals; triangles: Males; open circles: Females.

Melatonin in Gastrointestinal Tissue (Duodenum)
3-month old male rats (rattus norvegicus)



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CIRCADIAN AND CIRCASEMISEPTAN CHANGES IN CIRCULATING PROLACTIN IN RATS

György Nagy[1], Rita Jozsa[2], Germaine Cornélissen[3], Andras Olah[2], Michal Zeman[4], Jozsef Kaszaki[5], Valer Csernus[2], Weihong Pan[6], Franz Halberg[3]

[1]Semmelweis University of Medicine, Budapest, Hungary; [2]University Pecs, Hungary; [3]University of Minnesota, Minneapolis, MN, USA; [4]Comenius University, Bratislava, Slovakia; [5]University of Szeged, Hungary; [6]Pennington Biomedical Research Center, Baton Rouge, LA, USA

Supported by OTKA 043370, ETT 82/2003 (RJ), VEGA1/1294/04 (MZ)

Background

Animals were kept on two opposite regimens of 12 hours of light alternating with 12 hours of darkness (LD12:12, LD, and DL12:12 or reversed LD12:12, DL) in order to facilitate the sampling of blood during regular working hours for 7 consecutive days in a way to cover six timepoints at 4-hour intervals representing six different circadian stages in relation to light onset. The core temperature of a subsample of animals had been measured around the clock to check on the synchronization of the animals to their respective lighting regimen. The feasibility of using staggered lighting regimens was further assessed for the case of corticosterone assessed in the same animals by around-the-clock sampling from both rooms during the first 1.5 days. An about 4.3-day component was validated by nonlinear least squares for circulating corticosterone.

Aim

About-weekly (circaseptan) and half-weekly (circasemiseptan) changes have been documented for a host of variables. Whereas the circadian rhythm in prolactin is well known, this study aimed at assessing in rats any infradian variation along the 7-day scale.

Materials and methods

The data were analyzed by single and multiple cosinor. In view of the detection of a sex difference in prolactin, the data were expressed as a percentage of the mean value computed separately for males and females, so that the relative data could be pooled for a global analysis. Parameter tests served to examine any sex difference.

Results

A difference in circulating prolactin is observed between male and female rats, females having higher concentrations than males. The difference is statistically significant in both rooms kept on opposite lighting regimens (LD12:12: 158.4 ± 38.4 vs. 48.3 ± 4.1 ng/ml,

t=2.852, P=0.006; DL12:12: 124.4 ± 17.3 vs. 50.8 ± 4.4 ng/ml, t=4.204, P<0.001). Mean values do not differ with statistical significance between the two rooms for males or females. Whereas the circadian variation accounts for most of the variance (10%, P=0.079) in female rats, it is not the case for male rats. The males are characterized by a statistically significant 12-hour component accounting for 16% of the variance (P=0.016). Parameter tests confirm the difference in MESOR (F=20.132, P<0.001) and reveal a statistically significant difference in 24-hour amplitude as well (F=4.440, P=0.040). Analyses of pooled data are performed on relative data, expressed as a percentage of the mean values calculated separately for male and female rats. Least squares spectra of the pooled relative data in the frequency range of one cycle per week to two cycles per day indicate the presence of a circadian rhythm accounting for 6% of the overall variability, whereas the half-weekly component constitutes a small spectral peak. Further modeling of the data yields a composite model consisting of cosine curves with periods of 3.5 days (P=0.278) and 24 hours (P=0.038), accounting for 8% of the variance (P=0.075). A similar model is obtained for the log10-transformed data, accounting for 7% of the variance (P=0.105), where the 3.5-day component (P=0.140) and the 24-hour component (P=0.113) do not quite reach borderline statistical significance.

Discussion and Conclusion

By comparison with corticosterone, the prominence of both circadian and infradian components is much less. Apart from a sex difference in the MESOR of prolactin, this study shows a difference in the time structure of prolactin between male and female rats as well, males being characterized by a circasemidian component whereas females are primarily circadian periodic.

VALIDATION OF EXCLUSIVE DAYTIME MURINE SAMPLING ON ANTIPHASIC LIGHTING REGIMENS BY CIRCADIAN RHYTHMIC CORE TEMPERATURE BEHAVIOR

Andras Olah[1], Rita Jozsa[1], Germaine Cornélissen[2], Michal Zeman[3], György Nagy[4], Jozsef Kaszaki[5], Valer Csernus*, Weihong Pan[6], Franz Halberg[2]

[1]Univ. Pecs, Hungary; [2]Univ. of Minnesota, Minneapolis, MN, USA; [3]Comenius Univ., Bratislava, Slovakia; [4]Semmelweis Univ. of Medicine, Budapest, Hungary; [5]Univ. of Szeged, Hungary; [6]Pennington Biomedical Research Ctr., Baton Rouge, LA, USA

Support: ETT 82/2003 (RJ), VEGA1/1294/04 (MZ)

Background

Animals were kept in two opposite lighting regimens (LD and DL) in order to facilitate the subsequent sampling of blood and tissues during regular working hours for a week, to represent six timepoints at 4-hour intervals to cover six circadian stages expressed in hours after light onset (HALO). The lighting regimen is a known powerful synchronizer of circadian rhythms, but the adjustment of the circadian acrophase to a new lighting regimen is not immediate and may be fully achieved after more weeks on the new schedule. Core temperature is also considered a good marker rhythm, serving as a guide and a check for other variables that were planned to be collected as part of this experiment.

Aims

To determine whether the circadian acrophase of rat's core temperature kept in a reverse lighting regimen consisting of 12 hours of light alternating with 12 hours of darkness (DL: reversed) coincided with that of rats kept in LD, and to determine whether any difference in the circadian variation of core temperature between male and female rats can be found.

Materials and methods

A random sample of 29 rats (13 males and 16 females) served to determine the extent of synchronization to each of two lighting regimens: 12L:12D (LD) (N=16) and 12D12L (DL reversed) (N=13). Core temperature was measured around the clock every 4 hours for 24 hours (7 samples, the first timepoint being repeated 24 hours later). The data from each rat were analyzed by single cosinor, involving the least squares fit of a 24-hour cosine curve to each data series, to yield estimates of the MESOR (midline estimating statistic of

rhythm, M, a rhythm-adjusted mean value), the double amplitude (2A, a measure of the extent of predictable change within a day), and the acrophase (a measure of the timing of overall high values recurring each day). Results were summarized by population-mean cosinor, separately for males and females in each lighting regimen. Parameter tests compared the circadian characteristics of male and female rats as well as rats of each sex kept in opposite lighting regimens. In order to test for an anticipated antiphase, 180 degrees (12 hours) were added to the acrophase of animals kept in DL before testing the equality of acrophases of rats kept in LD or DL.

Results

Whether the circadian rhythm in core temperature was tested using 6 or 7 timepoints (without or with the repeated first timepoint), a circadian rhythm could be demonstrated with statistical significance ($P<0.05$) in 7 out of 29 rats. On a group basis, the circadian rhythm was invariably demonstrated ($P<0.05$). Parameter tests find that female rats have a higher MESOR of core temperature than male rats, whether the analyses are based on 6 timepoints (LD: 37.2° vs. 36.8°, $F=3.189$, $P=0.096$; DL: 37.5° vs. 37.0°, $F=5.547$, $P=0.038$) or on 7 timepoints (LD: 37.2° vs. 36.7°, $F=4.170$, $P=0.060$; DL: 37.4° vs. 37.0°, $F=6.408$, $P=0.028$). A test of equality of acrophases is statistically significant for males ($F=5.812$, $P=0.035$) but not for females ($F=0.289$, $P=0.600$) when considering only 6 timepoints. When all 7 timepoints are considered, the test is not significant for both males and females, suggesting that the animals were synchronized to their respective lighting regimens. A small acrophase difference detected with 6 but not with 7 timepoints in the case only of male rats may be interpreted to indicate that females, with statistically significantly higher core temperature, adjust faster to the daily routine or are less disturbed by external noise than males.

Discussion and Conclusion

The around the clock measurements of a marker rhythm such as core temperature serve a useful purpose to ascertain that animals are properly synchronized prior to the start of a large experiment involving multiple sampling of blood and various organ tissues aiming at assessing not only the circadian but also the about- weekly and half-weekly components of variation. Being able to work during regular hours while still covering all circadian stages in relation to light onset is particularly important when experiments are not limited to the study of circadian rhythms but focus also on infradian changes requiring sampling to continue for several consecutive days. Such marker rhythms are of interest in the many studies that focus on sex difference.

EXTRACIRCADIAN VARIATION OF ENDOTHELIN-1 IN MURINE PLASMA AND PITUITARY AND HUMAN BLOOD

Rita Jozsa[1], Jozsef Kaszaki[2], Germaine Cornélissen[3], Andras Olah[1], György Nagy[4], Valer Csernus[1], Michal Zeman[5], Weihong Pan[6], Franz Halberg[3]

[1]University Pecs, Hungary; [2]University of Szeged, Hungary; [3]University of Minnesota, Minneapolis, MN, USA; [4]Semmelweis University of Medicine, Budapest, Hungary; [5]Comenius University, Bratislava, Slovakia; [6]Pennington Biomedical Research Center, Baton Rouge, LA, USA

Support: ETT 82/2003 (RJ), VEGA1/1294/04 (MZ)

Background

During the past 8 years, no circadian rhythm in ET-1 could be detected, while it was found earlier during the descending stage of the solar activity cycle.

Aim

To assess any cyclicity of endothelin-1 (ET 1) in plasma and pituitary of mature Wistar rats of both sexes, sampled from 09:00 to 01:00 on the next day at 2-h intervals housed in two rooms kept on antiphasic lighting regimens.

Materials and methods

Samples were taken from rats under a short ether anesthesia, blood by cardiac puncture (2 ml/animal) and mixed with an ice-cold solution containing 1% aprotinum, 0.01% EDTA in physiological saline. The blood was centrifuged (4000 rpm, 20 min) and sera were removed and stored at 20°C. After bleeding, the animals were decapitated and the pituitaries were removed and placed in dry ice. They were stored at 20°C until measurement. Endothelin 1 was measured from both blood samples and pituitaries using a commercial ELISA ET 1 kit (Biomedica, Hungary). Of the protocols provided with the kit, protocol A was used for the blood samples and protocol B for the pituitary samples. Analyses were by cosinor. A study of circadian variation in endothelial cell function in human health on 9 subjects, 20-41 years of age, was also meta-analyzed.

Results

A circadian rhythm could not be demonstrated by cosinor with the fit of a 24-h cosine curve, in keeping with earlier work published during the past 8 years in Italy and Austria on the circulating ET-1 of humans and in keeping with still earlier work in Russia on the population density of the ET-1-producing endotheliocytes in mouse pinnal dermis. A 4.8-hour change is associated with a P-value of 0.024 from the no-rhythm (zero-amplitude) test for the data on ET 1 in plasma. A statistically significant ultradian was not demonstrated for ET 1 in the pituitary, but ET 1 in the pituitary correlates with that of plasma ($r=0.376$; $P=0.084$); when an outlier is removed, the r is 0.649 ($P<0.001$). A 12-hour component is found with the meta-analysis of data from a recent publication (Clinical Science 2002; 102: 547-552).

Discussion

A time-structural, broader-than-circadian chronome view recently also led to ultradian prominence for some neuropeptides. Observations over 10 years ago by contrast to the last 8 years demonstrated a circadian rhythm for ET 1 in France and in Italy, in the latter country in the very laboratory where a rhythm was subsequently not found.

Conclusion

The results indicate the need for a systematic follow-up work on ET-1 on the same species and tissue with the same method in the same setting, so that any infradian modulation, perhaps with a circadecadal or multidecadal cycle of extracircadians and/or circadians, can eventually be ruled in or out.

AGE- AND GENDER-DEPENDENT EFFECT OF EXPOSURE TO A TV SCREEN ON URINARY MELATONIN IN CHILDREN

Roberto Salti[1], Stefano Stagil, Roberto Tarquini[1], Federico Perfetto[1], Germaine Cornélissen[2], Maurizio Severini[3], Massimo Loy[3], Franz Halberg[2]

[1]University of Florence, Florence, Italy; [2]University of Minnesota, Minneapolis, MN, USA; [3]Institute of Atmospheric Science and Climate, Rome, Italy

Background

An effect of exposure to a TV screen on the urinary excretion of melatonin has been observed in children. Melatonin is known to undergo drastic changes as a function of puberty. Accordingly, it was of interest to examine whether the changes in melatonin associated with exposure to a TV screen were dependent on gender and/or age.

Aim

To assess whether the lowering of urinary melatonin excretion associated with exposure to a TV screen in children depends on gender and/or age.

Subjects and methods

The study involves 74 children (51 without any problem) from two schools in Cavriglia (Tuscany), Italy. For one week, children were allowed to watch TV in the evening. They were asked to record from Monday to Saturday (6 days) the time they watched TV, at which distance from the TV set, and the size of the TV set. For the next week, the children were asked to abstain from watching TV. At the end of each week, 24-hour urine samples were collected for the determination of melatonin by RIA. Both the concentration of urinary melatonin excretion and the total melatonin content in urine were determined. Differences were computed between the two weeks for each child, as a gauge of the effect of exposure to a TV set. The Student t test was used to assess any gender difference in the effect. Linear regressions were used to assess any trend as a function of age.

Results

The change in urinary melatonin excretion between the two weeks is changing with age, the lowering in melatonin concentration observed during exposure to a TV screen being more pronounced among the younger children ($r=-0.403$, $P<0.001$). A similar effect is found for melatonin content ($r=-0.376$, $P=0.001$). When considering only the 51 children without any problem, the age dependence of the effect remains statistically significant (concentration: $r=-0.436$, $P=0.001$; content: $r=-0.378$, $P=0.006$). This effect is consistent among boys and girls for melatonin concentration (boys: $r=-0.391$, $P=0.039$; girls: $r=-0.513$, $P=0.012$), but not for melatonin content (boys: $r=-0.295$, $P=0.127$; girls: $r=-0.423$, $P=0.044$). A gender difference is also seen in the overall change in melatonin content between the two weeks (irrespective of age), exposure to a TV set being associated with a statistically significant decrease in melatonin content in boys ($P=0.023$) but not in girls ($P=0.296$), the difference in overall response being statistically significant (Student $t=3.156$, $P=0.003$).

Discussion and conclusion

The TV exposure effect on urinary melatonin of children is both gender- and age-dependent. Coordinated laboratory studies on melatonin excretion by tadpoles stunted in normal growth by TV exposure at different stages of maturation and on more children are indicated. The effect of the content of programs watched by the children may then be reduced. The melatonin lowering effect seen at younger ages becomes an increase after 10 or 11 years of age. This reversal of a TV exposure effect on melatonin in children coincides with the occurrence of puberty, a critical age when important changes also occur naturally as a function of sexual maturation. Further studies are warranted to investigate whether such an effect on melatonin in children reverses again since a predictive effect on melatonin has been found in adults. An approach by chronomics (focusing on 3 elements of time structures: rhythms, trends and chaos) is warranted, with control of the reduced physical activity and/or other lifestyle alterations related to watching TV.

AGE- AND GENDER-DEPENDENT EFFECT OF EXPOSURE TO A TV SCREEN ON URINARY MELATONIN IN CHILDREN

Roberto Salti[1], Stefano Stagi[1], Roberto Tarquini[1], Federico Perfetto[1], Germaine Cornélissen[2], Maurizio Severini[3], Massimo Loy[3], Franz Halberg[2]

[1]University of Florence, Florence, Italy; [2]University of Minnesota, Minneapolis, MN, USA; [3]Institute of Atmospheric Science and Climate, Rome, Italy

Background

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Aim

To assess whether the lowering of urinary melatonin excretion associated with exposure to a TV screen in children depends on gender and/or age.

Subjects and methods

The study involves 74 children (51 without any problem) from two schools in Cavriglia (Tuscany), Italy. For one week, children were allowed to watch TV in the evening. They were asked to record from Monday to Saturday (6 days) the time they watched TV, at which distance from the TV set, and the size of the TV set. For the next week, the children were asked to abstain from watching TV. At the end of each week, 24-hour urine samples were collected for the determination of melatonin by RIA. Both the concentration of urinary melatonin excretion and the total melatonin content in urine were determined. Differences were computed between the two weeks for each child, as a gauge of the effect of exposure to a TV set. The Student t test was used to assess any gender difference in the effect. Linear regressions were used to assess any trend as a function of age.

Results

The change in urinary melatonin excretion between the two weeks is changing with age, the lowering in melatonin concentration observed during exposure to a TV screen being more pronounced among the younger children ($r=-0.403$, $P<0.001$). A similar effect is found for melatonin content ($r=-0.376$, $P=0.001$). When considering only the 51 children without any problem, the age dependence of the effect remains statistically significant (concentration: $r=-0.436$, $P=0.001$; content: $r=-0.378$, $P=0.006$). This effect is consistent among boys and girls for melatonin concentration (boys: $r=-0.391$, $P=0.039$; girls: $r=-0.513$, $P=0.012$), but not for melatonin content (boys: $r=-0.295$, $P=0.127$; girls: $r=-0.423$, $P=0.044$). A gender difference is also seen in the overall change in melatonin content between the two weeks (irrespective of age), exposure to a TV set being associated with a statistically significant decrease in melatonin content in boys ($P=0.023$) but not in girls ($P=0.296$), the difference in overall response being statistically significant (Student $t=3.156$, $P=0.003$).

Discussion and conclusion

The TV exposure effect on urinary melatonin of children is both gender- and age-dependent. Coordinated laboratory studies on melatonin excretion by tadpoles stunted in normal growth by TV exposure at different stages of maturation and on more children are indicated. The effect of the content of programs watched by the children may then be reduced. The melatonin lowering effect seen at younger ages becomes an increase after 10 or 11 years of age. This reversal of a TV exposure effect on melatonin in children coincides with the occurrence of puberty, a critical age when important changes also occur naturally as a function of sexual maturation. Further studies are warranted to investigate whether such an effect on melatonin in children reverses again since a predictive effect on melatonin has been found in adults. An approach by chronomics (focusing on 3 elements of time structures: rhythms, trends and chaos) is warranted, with control of the reduced physical activity and/or other lifestyle alterations related to watching TV.

CIRCADIAN AND CIRCASEPTAN CHANGES IN CIRCULATING CORTICOSTERONE OF RATS

Valer Csernus[1], Rita Jozsa[1], Germaine Cornélissen[2], Michal Zeman[3], Andras Olah[1], György Nagy[4], Jozsef Kaszaki[5], Weihong Pan[6], Franz Halberg[2]

[1]Univ. Pecs, Hungary; [2]Univ. of Minnesota, Minneapolis, MN, USA; [3]Comenius Univ., Bratislava, Slovakia; [4]Semmelweis Univ. of Medicine, Budapest, Hungary; [5]Univ. of Szeged, Hungary; [6]Pennington Biomedical Research Ctr., Baton Rouge, LA, USA

Support: OTKA 043370, ETT 82/2003 (RJ); VEGA1/1294/04 (MZ)

Background

Animals were kept on two opposite regimens of 12 hours of light alternating with 12 hours of darkness (LD12:12, LD, and DL12:12 or reversed LD12:12, DL) in order to facilitate the sampling of blood during regular working hours for 7 consecutive days in a way to cover six timepoints at 4-hour intervals representing six different circadian stages in relation to light onset. The core temperature of a subsample of animals had been measured around the clock to check on the synchronization of the animals to their respective lighting regimen.

Aim

About-weekly (circaseptan) and half-weekly (circasemiseptan) changes have been documented for a host of variables. Whereas the circadian rhythm in corticosterone has been well known since 1958, this study aimed at questioning the variability in period length and thus the uncertainty of the circadian period by 6 replications, while also assessing any infradian variation in rats, assessable by sampling around the clock along a 7-day scale. To facilitate the practicability of the study, multiphasic lighting regimens were used during standardization and sampling.

Materials and methods

Corticosterone was determined by RIA. The data were analyzed by linear-nonlinear rhythmometry and by parameter tests.

Results

A circadian rhythm is demonstrated with statistical significance in male and female rats and overall ($P<0.001$). Parameter tests confirm the MESOR difference between males and females ($F=34.649$, $P<0.001$). Analyses of pooled data are thus performed on relative data (and their log10-transformation), expressed as a percentage of the mean values calculated separately for male and female rats.

Least squares spectra in the frequency range of one cycle per week to two cycles per day indicate the presence of a prominent circadian rhythm accounting for 40% of the overall variability as well as a statistically significant 12-hour component ($P=0.036$) qualifying the circadian waveform of corticosterone. Further modeling of the data yields a composite model consisting of cosine curves with periods of 7 days ($P=0.061$), 24 hours ($P<0.001$) and 12 hours ($P=0.004$), accounting for 49% of the variance ($P<0.001$). Similar analyses on the log10-transformed data account for 51% of the variance ($P<0.001$) when considering cosine curves with periods of 7 days ($P=0.038$), 3.5 days ($P=0.039$) and 1 day ($P<0.001$). Separate analyses of male and female rats indicate the presence of a prominent circadian rhythm ($P<0.001$) with the contribution to the waveform by the second harmonic with a period of 12 hours (Males: $P=0.048$; Females: $P=0.058$) together with a circaseptan component of borderline statistical significance for males ($P=0.057$) or a weak circasemiseptan component for females ($P=0.180$). The model accounts for 65% of the variance for males ($P<0.001$) and for 38% of the variance for females ($P=0.002$).

Nonlinear analyses of the pooled log10-transformed (relative) data confirm the presence of both the circadian rhythm, with an estimated period of 24.03 hours (95% CI: 23.30-24.75 hours), and an infradian component with an estimated period of 103.8 hours (4.3 days) (95% CI: 79.5-128.1 hours). These results are similar for male and female rats: for males, the circadian and infradian periods are estimated as 24.00 (23.20-24.79) and 108.2 (61.2-155.1) hours; for females, the respective periods are estimated as 24.03 (22.57-25.49) and 101.9 (71.5-132.3) hours.

Discussion and Conclusion

This study shows the feasibility of assessing multi-frequency components in the laboratory based on sampling restricted to the usual working hours by housing animals in staggered lighting regimens. Such a protocol is of particular interest when sampling is prolonged over several consecutive days to determine infradian components, as done herein. Major findings of this study are: 1. a sex difference, female rats having higher circulating corticosterone concentrations than male rats; and 2. the demonstration of an infradian component of about 4.3 days found consistently in both male and female rats together with a prominent circadian rhythm.

THE RELATIONSHIP BETWEEN AGE AND CIRCADIAN BLOOD PRESSURE VARIATION

Siegelová J., Dušek J., Fišer B., Homolka P., Vank P., Vlček J., Cornelissen G.*, Halberg F*.

Department of Functional Diagnostics and Rehabilitation, Masaryk University Brno, St. Anna Faculty Hospital Brno, Czech Republic, *University of Minnesota, USA

Introduction

Time structures in us and around us are studied in chronobiology.. Most if not all components of variation found in biota are also found in the environment, and vice versa [1]. For instance, about daily changes are seen in almost every biological variable under 24-hour synchronized conditions. It has also long been known that the phase of circadian rhythms can be manipulated by changing the phase of the environmental cycles [2]. At least for the case of circadian rhythms, their genetic inheritance has been demonstrated on a molecular basis [3, 4], suggesting that the influence from the environment has been acquired genetically during the course of evolution.

Aim of study

The relationship between age and circadian blood pressure (BP) variation in man was the aim of the present study.

Subjects

One hundred eight seven subjects (130 males, 57 females), 20-77 years old, were recruited for seven-day BP monitoring.

Methods

Colin medical instruments (Komaki, Japan) were used for ambulatory BP monitoring (oscillation method, 30 minutes interval between measurements). Sinusoidal curve was fitted (minimum square method) and mean value and amplitude of the curve (double amplitude corresponds to the night-day difference) were evaluated every day of monitoring. Average 7 day values of the mean (M) and of double amplitude (2A) for systolic BP (SBP), diastolic BP (DBP) and heart rate (HR) were determined in each subject.

Results

Mean values of M (±SD) for the whole group were: SBP- 127±8, DBP - 79±6 mmHg, HR - 70±6 bpm; of 2A: SBP - 21±7, DBP - 15±5 mmHg, HR - 15±6 bpm (Fig. 1, 2, 3, 4, 5, 6). The linear relationship between M of SBP and age ($r=0.341$, $p< 0.001$) and DBP and age ($r=0.384$, $p<0.001$) was found (difference between 20 and 77 years: SBP - 16, DBP-12 mmHg). 2A of SBP and DBP was increasing with age till 35 years, then the curve remained relatively flat till 55 years (maximum at 45 years) and then decreased again (difference between 45 and 77 years: SBP- 13mmHg, DBP-12 mmHg). Heart rate M and 2A were age-independent.

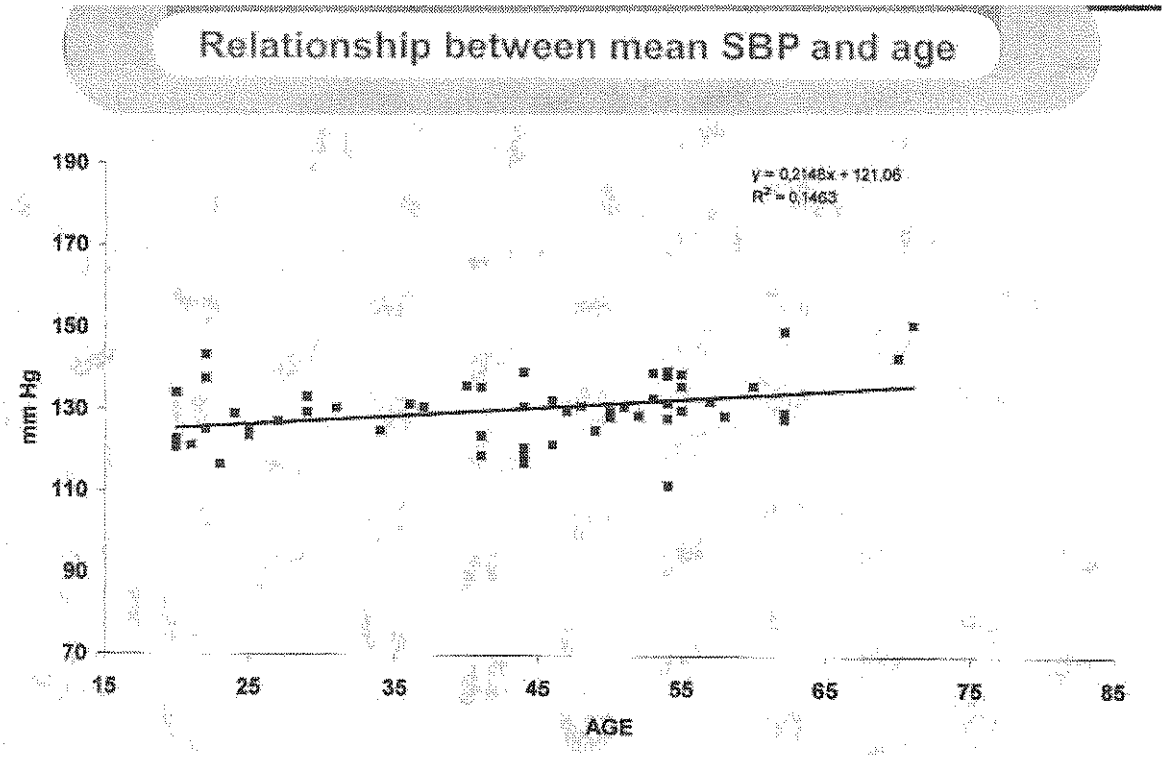

Fig. 1

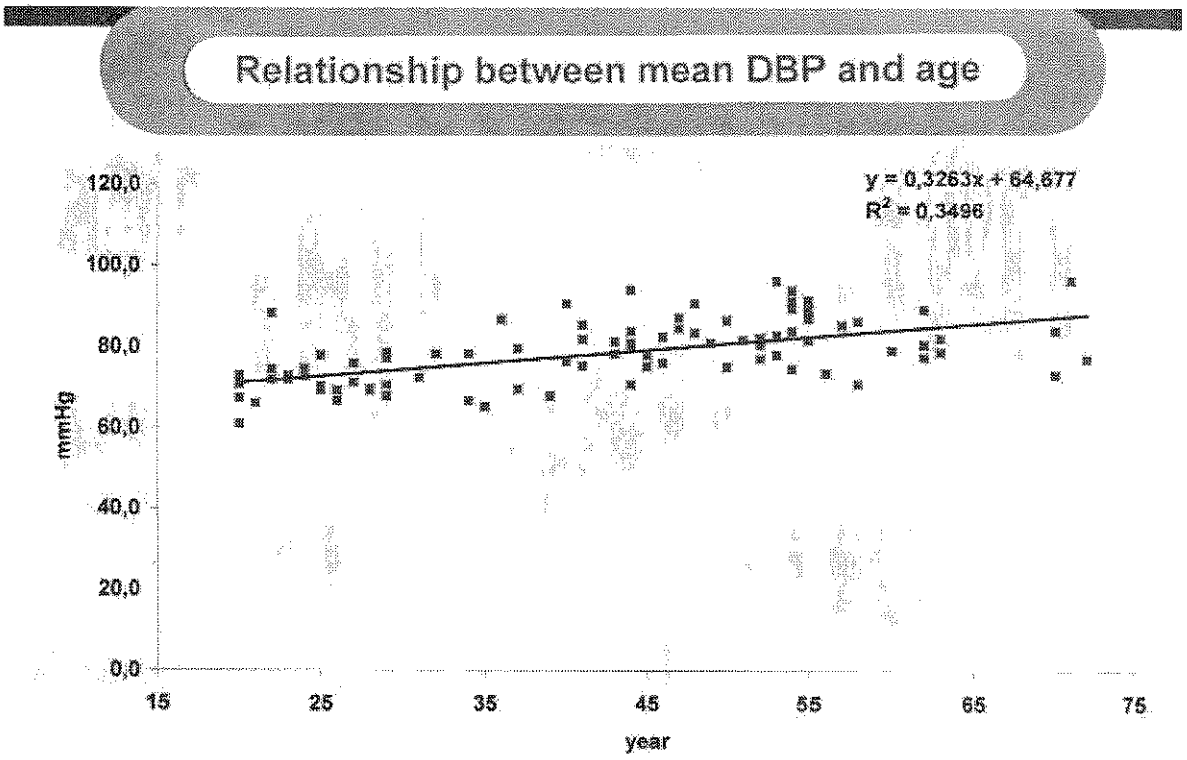
Relationship between mean DBP and age
y = 0,3263x + 64,677
R² = 0,3496
120,0
100,0
80,0
60,0
40,0
20,0
0,0
mmHg
15
25
35
45
55
65
75
year

Fig. 2

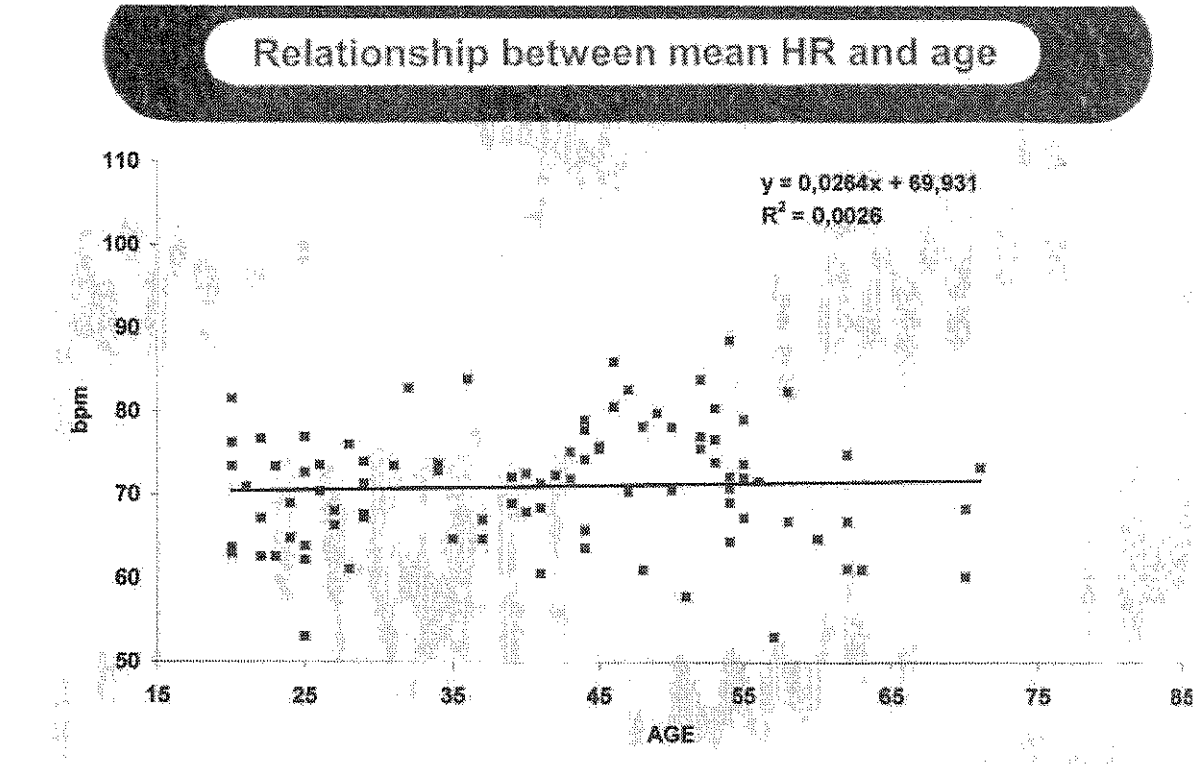
Relationship between mean HR and age
y = 0,0264x + 69,931
R² = 0,0026
110
100
90
80
70
60
50
bpm
15
25
35
45
55
65
75
85
AGE

Fig. 3

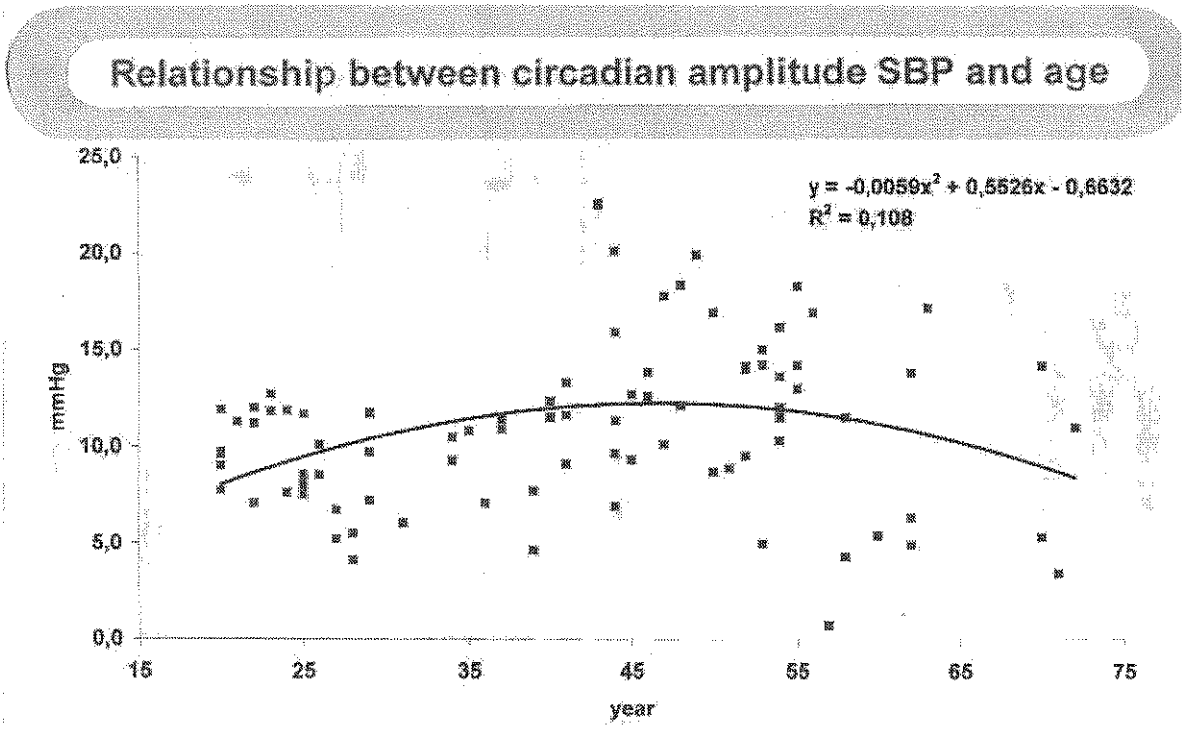
Relationship between circadian amplitude SBP and age
y = -0,0059x² + 0,5526x - 0,6632
R² = 0,108
mmHg
year
25,0
20,0
15,0
10,0
5,0
0,0
15
25
35
45
55
65
75

Fig. 4

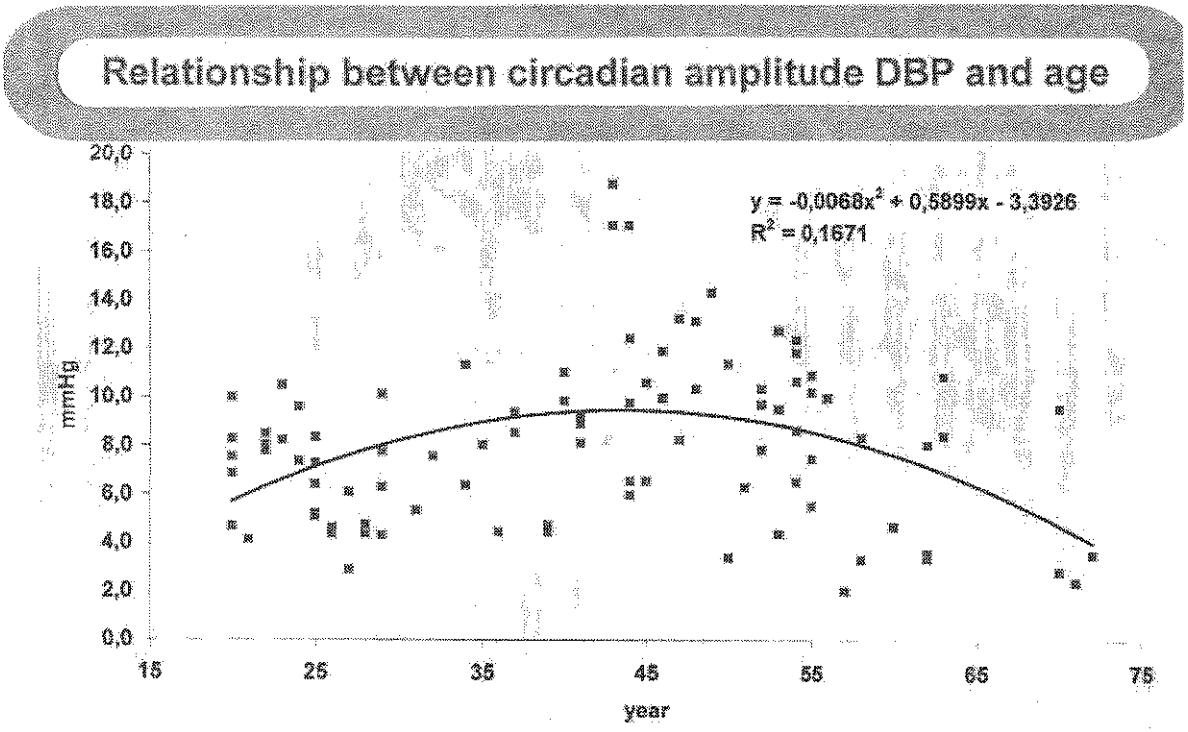
Relationship between circadian amplitude DBP and age
y = -0,0068x² + 0,5899x - 3,3926
R² = 0,1671
mmHg
year
20,0
18,0
16,0
14,0
12,0
10,0
8,0
6,0
4,0
2,0
0,0
15
25
35
45
55
65
75

Fig. 5

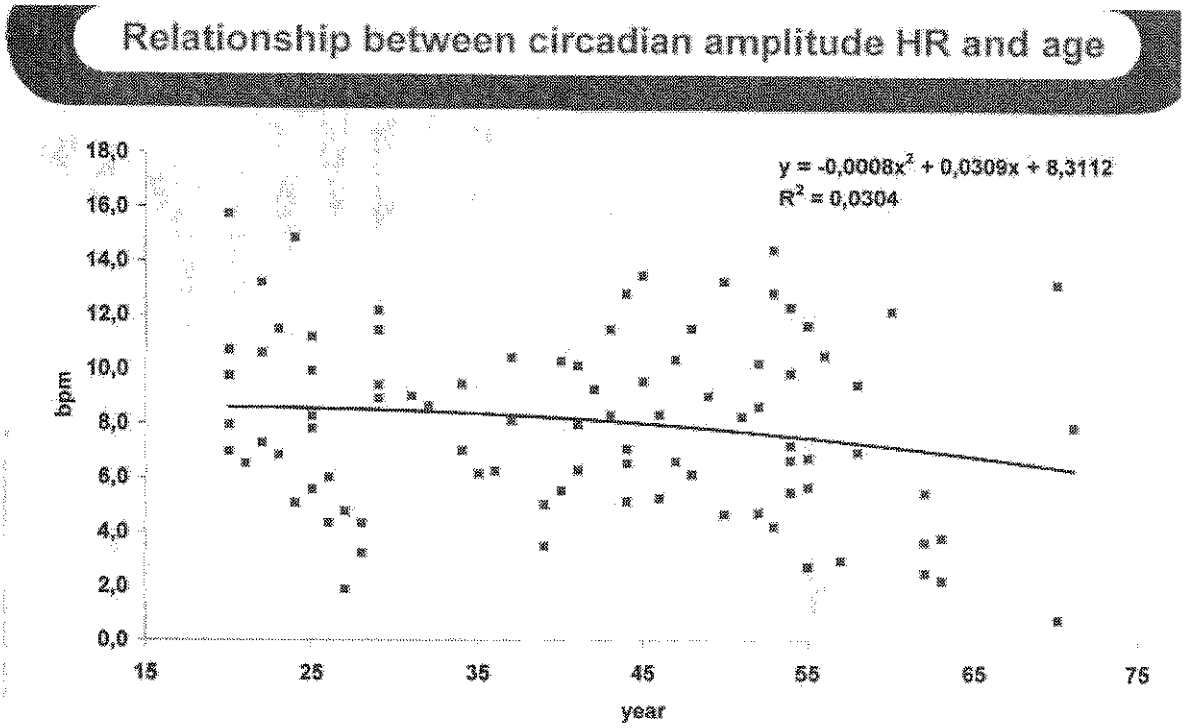

Fig 6

Discussion

The guidelines for antihypertensive therapy are based on casual blood pressure measurement in the doctor office. The variation of blood pressure in individual patients decrease the sensitivity and specificity of this approach. It is self- evident that 24 hour ambulatory monitoring of blood pressure is better. However the data of 24 hour ambulatory monitoring are also not representative. Blood pressure record of 7 day measurement showed that the circadian variability changes from day to day and 24 hour record is not long enough. Seven day monitoring of blood pressure is preferable and can reflect the differences between working days and weekend. Seven day monitoring enables also to determine the amplitude of circaseptan (one week) rhythm. Circaseptan rhythm is inborn. We have demonstrated this fact by monitoring the 86 premature newborns babies and we could made the following conclusions:

Slow significant oscillations ($p<0.05$) with a different period between 5 to 10 days (an about-weekly component, circaseptan) were found either in HR, SBP or DBP in all newborn babies (100 percent). The significant peak of circadian rhythm ($p<0.05$) was found in HR in 50 percent of premature newborn babies, in SBP or DBP only in 43 percent. The frequency peaks of circadian rhythm in cardiovascular parameters were smaller than the frequency peaks of circaseptan rhythm. Cosinor analysis showed that circaseptan rhythms are more prominent in blood pressure and heart rate than the circadian rhythms. Our results gave us the opportunity to analyze the dependence of the circaseptan rhythm in blood pressure and heart rate on the different days of the week. Our results showed that the circaseptan rhythms in blood pressure and heart rate are

independent of the week days and the origin of the circaseptan rhythm is dependent on the time of birth (5).

The analysis of circadian amplitude is very important. Watanabe and Halberg found significant increase of risk of cardiovascular morbidity (6).

The analysis of 48hour blood pressure data have led to the identification of new disease risk syndrome CHAT (circadian hyper-amplitude-tension, a condition characterized by a circadian amplitude exceeding the upper 95% prediction limit)., Together with an excessive pulse pressure (above 60 mmHg), CHAT and deacreased hear rate variability can make the difference between <4% and 100% morbidity in a 6-year prospective study (7).

Comparison of circadian characteristics from day to day in records spanning at least two days further indicates the shortcomings of monitoring limited to a single 24-hour span (6,8,9). Prolonging the monitoring from one to two days reduces the uncertainty in the estimation of circadian parameters by about 35% (10), whereas further information on the biological week (11,12,13,14) requires monitoring for at least 7 days, the current recommendation of BIOCOS for everybody at the outset (15).

Conclusion

Mean values of SBP and DBP were increasing with age till 75 years, but night-day difference of SBP and DBP reached maximum value at 45 years and then decreased.

Support: MSM 141100004.

REFERENCES

1. Halberg F, Cornélissen G, Otsuka K, Watanabe Y, Delyukov A, Gorgo Y, Zhao ZY, Weydahl A, Sothern RB, Siegelova J, Fiser B, Dusek J, Syutkina EV, Perfetto F, Tarquini R, Singh RB, Rhees B, Lofstrom D., Lofstrom P, Sonkowsky R, Johnson PWC, Schwartzkopff O, International BIOCOS Study Group. Cross-spectrally coherent ~10.5- and 21-year biological and physical cycles, magnetic storms and myocardial infarctions. Neuroendocrinoogy Letters 2000; 21: 233-258.
2. Halberg F. Chronobiology. Annual Reviews of Physiology 1969; 31: 675-725.
3. Pennisi E. Multiple clocks keep time in fruit fly tissues. Science 1997; 278: 1560-1561.
4. Plautz JD, Kaneko M, Hall JC, Kay SA. Independent photoreceptive circadian clocks throughout *Drosophila*. Science 1997; 278: 1632-1635.
5. Siegelová j., Cornelissen G., Schwarzkopff O.,Halberg F. Time structures in the development of children.Neuroendocrinol.Lett 2003, 24(Suppl.1): 126-131.
6. Watanabe Y, Cornélissen G, Halberg F, Bingham C, Siegelova J, Otsuka K, Kikuchi T. Incidence pattern and treatment of a clinical entity, overswinging or circadian hyperamplitudetension (CHAT). Scripta medica 1997; 70: 245-261.
7. Cornélissen G, Otsuka K, Chen CH-H, Halberg F. Circadian Hyper-Amplitude-Tension (CHAT) and an elevated pulse pressure are separate cardiovascular disease risk factors. Clin Exp Hypertens, in press.
8. Cornélissen G. Instrumentation and data analysis methods needed for blood pressure monitoring in chronobiology. In: Scheving LE, Halberg F, Ehret CF (eds). Chronobiotechnology and Chronobiological Engineering. Dordrecht, The Netherlands: Martinus Nijhoff, 1987, pp. 241-261.

9. Tamura K, Wu J, Cornélissen G, Halberg F. Agreement between consecutive ambulatory 24-hour blood pressure and heart rate pro?les in Japanese hospital staff. Progress in Clinical and Biological Research 1990; 341A: 263-272.
10. Halberg F, Scheving LE, Lucas E, Cornélissen G, Sothern RB, Halberg E, Halberg J, Halberg Francine, Carte J Jr, Straub KD, Redmond DP. Chronobiology of human blood pressure in the light of static (room-restricted) automatic monitoring. Chronobiologia 1984; 11: 217-247.
11. Carandente F, Cornélissen G, Halberg F Further data and analyses. Chronobiologia 1994; 21: 311-314.
12. Halberg F, Cornelissen G, Raab F, Schaffer E, Siegelova J, Fiser B, Dusek J, Prikryl P, Otsuka K. Automatic physiologic 7-day monitoring and chronobiology. Japanese J of Electrocardiology 1995; 15 (Suppl. 1): S-1-5 - S-1-11.
13. Siegelova J, Homolka P, Dusek J, Fiser B, Cornélissen G, Halberg F. Extracircadian-to-circadian variance transpositions early and vice versa late in life in the human circulation. Proceedings, 1st International Symposium, Workshop on Chronoastrobiology & Chronotherapy (Satellite Symposium, 7th Annual Meeting, Japanese Society for Chronobiology), Kudan, Chiyodaku, Tokyo, 11 Nov 2000, pp. 58-60.
14. Singh RB, Cornélissen G, Siegelova J, Homolka P, Halberg F. About half-weekly (circasemiseptan) pattern of blood pressure and heart rate in men and women of India. Scripta medica 2002; 75: 125-128.
15. Halberg F, Smith HN, Cornélissen G, Delmore P, Schwartzkopff O, International BIOCOS Group. Hurdles to asepsis, universal literacy, and chronobiology-all to be overcome. Neuroendocrinology Letters 2000; 21: 145-160.

EFFECT OF COMBINED EXERCISE TRAINING ON HEART RATE VARIABILITY IN CHRONIC HEART FAILURE

Jančík J.[1], Siegelová J.[1], Dobšák P.[1], Svačinová H.[1], Placheta Z.[1], Fišer B.[2], Dušek J.[1], Eicher J.C.[4], Kožantová L.[1], Mífková L.[1], Chludilová V. [1], Vohlídalová I.[1], Sosíková M. [1],Vítovec J.[3]

[1] Department of Functional Diagnostics and Rehabilitation, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[2] Institute of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[3] Ist Department of Cardioangiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[4] Department of Cardiology, Hôpital du Bocage and University of Burgundy, Dijon, France

INTRODUCTION

Low heart rate variability (HRV) is an indicator of increased risk of sudden cardiac death after myocardial infarction in patients with chronic heart failure (CHF) (*1, 2*). It is supposed that regular physical activity could modify this risk factor; however, most of the training methods including the rehabilitation protocols used also in our previously published studies are based on aerobic exercise (*3, 4*), and until now there is lack of valid information from actual bibliography concerning the effects of resistance or combined long-term lasting training on the heart rate variability parameters (HRV). Classical methodology to evaluate the benefit of physical training is based on cardiopulmonary exercise testing. However, this examination is time consuming and could be potentially life-threatening. In contrast, the examination of HRV is comfortable for the patient and without any risk. The aim of this study was to investigate if HRV testing can be useful for determination of the influence of 8-weeks combined exercise training on the heart rate variability expressed in frequency domain parameters in group of patients with CHF.

PATIENTS AND METHODS

Ten patients (mean body weight 85 ± 12 kg; mean age 61 ± 13 years) with chronic heart failure were selected according to the inclusion criteria listed below. The treatment regimen of all selected patients was optimised to ensure that the patients were symptomatically stable. Standardised pharmacological treatment at the beginning and the end of the 8-week period included administration of angiotensin-converting enzyme inhibitors (ACEI), ß-blockers, diuretics and digitalis in varying combinations. Inclusion criteria were as follows: age over 18 years, symptomatic chronic congestive heart failure

NYHA class II-III determined for at least 3 months, and stable for at least 6 weeks; left ventricular ejection fraction <40 % (assessed by 2D-echocardiography); valid cardiopulmonary exercise testing limited by dyspnea or fatigue (wihout chest pain), with a symptom-limited oxygen consumption (VO2SL) < 20 ml.kg-1.min-1. Before the inclusion to the study all the subjects signed the Informed Patient's Consent; the study was approved by the local Ethical Committee and conforms to the principles outlined in the Declaration of Helsinki and to GCP guidelines of European Community.

Heart rate variability was registered by the system Varia-Pulse TF-3. A short-time evaluation of the heart rate variability was done using the beat-to-beat non-invasive monitoring of ECG, spontaneous and metronome-controlled breathing at 0.33Hz before and after the training period of 8 weeks. HRV frequency-domain parameters were determined by spectral analysis of pulse interval (PI).

Combined exercise training was realized at the Department of Functional Diagnostics and Rehabilitation 3 times a week. Resistance training elements were included into the exercise protocol only after 2 weeks of aerobic training. One exercise session lasted 60 min, and included: warm-up period (10 min), period of aerobic exercise on bicycle ergometer with load intensity at the level of anaerobic threshold (20 min), period of fitness (or resistance) training performed on combined training machine (20 min), and relaxation period (10 min). In the period of fitness (resistance) training all the subjects started to exercise at 30% level of 1-RM (one repetition maximum), and after 2 weeks they continued at 60% level of 1-RM The rehabilitation programme was performed by the patients for eight weeks.

Standard exercise spiroergometry (Blood Gas Analyser, MedGraphics, USA) up to the maximum limited by symptoms was carried out before and after 8 weeks of training to assess symptom-limited oxygen consumption (VO_{2SL}), maximal workload (W_{max}), metabolic equivalents (MET_S) and maximal heart rate (HR_{max}). The first spiroergometry test was applied also to determine the anaerobic threshold (ANP) in order to decide the individual training intensity.

Statistical analysis of functional data was performed using the Wilcoxon paired test, the Chi-2 test, the Friedmann test, and analysis of variance ANOVA. The P value < 0.05 was considered as significant.

RESULTS

Control spiroergometry testing after the 8 weeks of training showed significant increase of W_{max} (110 ± 20 W; $^*P < 0.05$), and also a significant increase of VO_{2SL} values (1551 ± 261 ml O_2 .min-1; *P < 0.05); the increase of both values was approximately +20 % compared to the initial values. Table 1 summarizes the results of all evaluated functional parameters.

Table 1

Results of evaluated functional parameters assessed by bicycle spiroergometry testing (results are expressed as mean ± SD).

Functional parameters	W_{max} (W)	$W_{max}.kg^{-1}$ ($W.kg^{-1}$)	VO_{2SL} ($mlO_2.min^{-1}$)	$VO_{2SL}.kg^{-1}$ ($mlO_2.min^{-1}.kg^{-1}$)	METs
before	93 (± 17)	1.0 (± 0.2)	1399 (± 284)	15.2 (± 3.4)	4.3 (± 1.0)
after	110 (± 20)	*** 1.2** (± 0.2)	*** 1551** (± 261)	*** 16.9** (± 2.8)	*** 4.7** (± 0.9)

W_{max} – maximal workload; $W_{max}.kg^{-1}$ – maximal workload per kg; VO_{2SL} – symptom-limited oxygen uptake; $VO_{2SL}.kg^{-1}$ – symptom-limited oxygen uptake per kg; METs – metabolic equivalents; * $P < 0.05$

Frequency-domain HRV parameters were evaluated as follows: the width of pulse intervals (PI; ms), the variance of R-R intervals over the selected time interval (or total power - TP; ms^2), the power of low frequency (0.04-014 Hz) component (LF; ms^2), the power of high frequency (0.15-04 Hz) component (HF; ms^2) and ratio of LF power to HF power (LF/HF). Spectral analysis revealed a significant increase of the total power (TP) of HRV after 8 weeks of combined exercise training (2829 ± 3000 ms^2; * $P < 0.05$) in comparison with the TP initial values. An increase of other HRV parameters was also observed but without statistical significance.

Table 2

Results of the spectral analysis of registered HRV frequency-domain parameters (results are expressed as mean SD).

Functional parameters	PI (ms)	TP (ms^2)	LF (ms^2)	HF (ms^2)	LF/HF
before	1016 (± 137)	891 (± 1011)	191 (± 178)	665 (± 820)	1.01 (± 1.11)
after	1046 (± 124)	*** 2829** (± 3000)	256 (± 214)	2573 (± 2875)	0.80 (± 1.12)

PI – pulse interval; TP – total power; LF – low-frequency component, HF – high-frequency component; LF/HF – LF to HF ratio; * $P < 0.05$

The results of HRV spectral analysis and the results of functional performance testing indicate that HRV parameters could be useful for the evaluation of the effectiveness of physical training.

DISCUSSION

In the last 20 years the decreased heart rate variability has been shown to be a significant sign of sudden death risk in patients after myocardial infarction (*5*), and the predictive value of decreased heart rate variability is comparable to the ejection fraction volume in the risk stratification of patients after myocardial infarction (*6*). The autonomic nervous system is permanently influenced by a variety of stimuli from inner or outer origin. Age and health status belong to the inner stimuli, whereas climatic conditions, day (night) period, actual psychic and physic workload, or changes of the body position are the stimuli of outer origin (*7*). For easier interpretation of the results of HRV examination a test (supine - standing - supine position) was introduced in which the vagal activity increases in supine position, whereas the sympathetic tone is increased in standing position. Moreover, after repeated supine position an overshoot of the spectral power of the high frequency component of the heart rate spectral analysis appears. Thus, in order to analyze the data of vagal acitivity with maximal precision, an analysis of spectral parameters after repeated supine position is recommended (*8*). Regarding to the fact that the amplitude of respiration arrhythmia is predominantly dependent on the frequency and depth of breathing (without breathing frequency control), the variability at high frequency can be submitted to non-predictable changes. A deep and slowed respiration to 6 breaths cycles per minute shifts the top of respiratory spectra in the area of 0.1Hz and so can imitate an increase of sympathetic tone modulation of cardiac rhythm (*9*). In our present study we evaluated 5min intervals of the HRV using metronome-controlled breathing at 0.33Hz. The adaptation of cardiovascular system on resistance exercise training is different from the adaptation on dynamic exercise training. Heart muscle in resistance training shows signs of concentric hypertrophy, whereas the heart muscle adaptation in dynamic training is characterized by increase of heart cavities and only limited heart wall thickening. In that case the hypertrophy is considered as eccentric (*10*). In contrast to the resistance exercise the aerobic (or dynamic) training is more efficient on the decrease of heart rate and systolic blood pressure at rest, and also on the increase of stroke volume at rest and during exercise (*11*). Thus, it is possible to suppose that various types of exercise influence the HRV from different manner. The results of our study showed the statistically significant difference of TP.

Up to the present the influence of combined training on the patients' performance and the autonomic nervous system has not been fully explained. Our results have shown that 8 weeks of combined training led to the increase of functional capacity in patients with chronic heart failure and the increase of total spectral power. This study contributes to the knowledge about rehabilitation training importance in patients with chronic heart failure.

Acknowledgement

This study was supported by the grant of Czech Ministry of Health IGA MZČR, No. NR 7983-3, 2004.

Abbreviations used

CHF – chronic heart failure, HF – high-frequency component, HR_{max} – maximal heart rate, HRV – heart rate variability, NYHA – New York Heart Association, LF – low-frequency component, PI – pulse interval; LF/HF – LF to HF ratio, VO_{2SL} – symptom-limited oxygen uptake, VO_{2AT} – oxygen uptake at anaerobic threshold, TP – total power, W_{max} - maximal workload

REFERENCES

1. La Rovere MT, Bigger JT, Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. Lancet 1998; 351: 478-484.
2. Galinier M, Pathak A, Fourcade J. et al. Depressed low frequency power of heart rate variability as an independent predictor of sudden death in chronic heart failure. Eur Heart J 2000; 21: 475-482
3. Jančík J, Várnayová L, Siegelová J. et al. Heart rate variability in patients with chronic ischemic heart disease: effect of 8-week exercise training. Proceedings of Symposium: The Importance of Chronobiology in Diagnosing and Therapy of Internal Diseases. F.Halberg, T.Kenner, B.Fišer (eds.). IDVPZ Brno 2002: 179-184.
4. Jančík, J., Siegelová, J., Dobšák, P. et al. Baroreflex sensitivity and heart rate variability in patients with chronic ischemic heart disease and systolic dysfunction: effect of exercise training. Clin Autonom Res 2003; 13(1): 57.
5. Hohnloser SH, Klingenhebel T, Zabel M. Identification of patients after myocardial infarction at risk of life-threatening arrhythmias. Eur Heart J 1999; Suppl.1: 11-20.
6. Halámek J, Kára T, Jurák P. et al. Variability of phase shift between blood pressure and heart rate fluctuations - A marker of short-term circulation control. Circulation 2003; 108(3): 292-297.
7. Semrád B, Fišer J, Honzíková N. Aging and cardiac autonomic status. In: Clinical Guide to cardiac autonomic test. M. Malik (ed.). London, Kluwer Academic Publishers 1998: 285-300.
8. Opavský J. Metody vyšetřování autonomního nervového systému a spektrální analýza variability srdeční frekvence v klinické praxi. In: J.Salinger: Variabilita srdeční frekvence a její hodnocení v biomedicínských oborech - od teorie ke klinické praxi. Sborník článků a abstrakt. Univerzita Palackého v Olomouci. Olomouc, 2004. ISBN 80-244-0805-8
9. Kautzner J, Malik M. Variabilita srdečního rytmu a její klinická použitelnost. Cor Vasa, 1998: 40(5): 244 - 251.
10. Máček M, Máčková J. Fyziologie tělesných cvičení. Sdružení pro rozvoj zdravotní a tělesné výchovy ve spolupráci s nakladatelstvím ONYX Praha, 1995. ISBN 80-85228-20-3
11. Pollock ML, Franklin BA, Balady GJ et al. Resistance exercise in individuals with and without cardiovascular disease. Benefits, rationale, safety, and prescription. An advisory from the Committee on Exercise, Rehabilitation, and Prevention, Council on Clinical Cardiology, American Heart Association. Circulation 2000; 101: 828-833.

REHABILITATION IN CHRONIC CONGESTIVE HEART FAILURE

Eicher J.C.[1], Dobšák P. [2], Berteau O.[1], Walker P.[3], Vergés B.[4], Maillefert J.F.[4], Casillas J.M.[4], Brunotte F.[3], Louis P.[1], Wolf J.E.[1] Siegelová J.[2], Fišer B. [2], Jančík J. [2], Vank P. [2], Svačinová H. [2]

[1] Department of Cardiology, Hôpital du Bocage and University of Burgundy, Dijon, France
[2] Department of Functional Diagnostics and Rehabilitation, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[3] Department of Nuclear Medicine, Hôpital du Bocage and University of Burgundy, Dijon, France
[4] Department of Rehabilitation, Hôpital du Bocage and University of Burgundy, Dijon, France

INTRODUCTION

Chronic heart failure is a complex metabolic syndrome with impaired left ventricular function and poor prognosis (*1*). Several studies reported strong rise of sympathetic activity, the onset of peripheral vascular remodeling and strength muscle metabolic alterations in response to exercise (*2*); exercise intolerance in CHF is mainly due to muscle deconditioning (*3, 4*). The exercise training has been shown to improve the functional capacity, quality of life and also the patterns of strength muscles, and therefore should be considered as an integral part of therapeutic standards in such patients. (*5-7*). However, even rehabilitation based on physical exercise can improve both exercise capacity and symptoms, some patients may be too ill to exercise. Several studies reported the increase of oxidative capacity in skeletal muscle fibers, of enhancement of muscular regeneration and of the atrophy prevention by low-frequency electrical stimulation (ES) of strength muscles (*8-10*). Neverteheless, until now the muscle reconditioning by electrical stimulation (ES) has been evaluated in few small series, and the effects of this technique compared to classical training in a randomized trial has been poorly evaluated.

PATIENTS AND METHODS

The study population comprised twenty-four patients (5 women, 19 men, mean age 54 ± 9 years) with stable, chronic congestive heart failure. Four patients were in NYHA functional class II, 19 of them were in class III, and one was in class IV. The etiology of CHF was idiopathic dilated cardiomyopathy (17 subjects), ischemic heart disease (6 subjects), and valvular heart disease (1 subject). All patients gave informed consent. Patients were randomized to enter either a classical bicycle training program, or an electrical stimulation program. Patients in the bicycle group (group 1) underwent 25 daily sessions of 20 minutes bicycle exercise, at 60-80% of their maximal heart rate. In the electrical stimulation group (group 2), low-frequency (10 Hz) ES was applied to both

quadriceps and calf muscles, using ELPHA 2000 stimulators (Danmeter A/S, Odense, Denmark) programmed alternately 20 seconds on and 20 seconds off. Twenty five daily ES sessions of 1 hour were achieved.

The following parameters were collected before and at the end of the rehabilitation program:

- distance walked in 6 minutes
- during bicycle exercise testing with gas exchange analysis:
- VO_{2SL} (symptom-limited VO^2)
  - VO_{2AT} (VO^2 at anaerobic threshold)
  - exercise duration
  - W_{max} (maximal workload)
  - HR_{max} (maximal heart rate)
- using Doppler study of the common femoral artery:
  - mean velocity at rest
  - mean velocity after 15 minutes of electrically induced muscle exercise

Statistical analysis was performed using the Wilcoxon paired test, the Chi-2 test and the Friedmann test. The P value < 0.05 was considered as significant.

RESULTS

- Distance walked in 6 minutes improved significantly in both groups. It improved more in the ES group (+ 72m) than in the bicycle group (+ 29m; *Tables 1, 2 and 3*).
- VO_{2SL} improved significantly, either by ES (+ 6%) or by bicycle training (+ 8%). The difference between groups is not statistically significant (*Tables 1, 2 and 3*).
- VO_{2AT} improved non-significantly in the ES group (+ 10%), and significantly (+ 19%) in the bicycle group. The difference between groups is significant (*Tables 1, 2 and 3*).
- Similarly, exercise duration increased non-significantly in the ES group (+ 31s), and significantly in the bicycle group (+ 82s - *Tables 1, 2 and 3*).
- Maximal achieved workload increased significantly in both groups, as did maximal heart rate (HR_{max}). The difference between groups is non-significant (*Tables 1, 2 and 3*).

Table 1
Values of parameters in the "bicycle" group before and after 5 weeks of exercise training.

Bicycle group (group 1)	**pre**	**post**	***P* value**
VO_{2SL} ($ml.kg^{-1}.min^{-1}$)	17.28	***18.83**	< 0.03
VO_{2AT} ($ml.kg^{-1}.min^{-1}$)	11.32	***13.51**	< 0.001
W_{max} (W)	96.67	***109.7**	< 0.02
6-min walking test (m)	468.09	***497.09**	< 0.005
Exercise duration (s)	545	***627**	< 0.01
HR_{max} ($beats.min^{-1}$)	144	***154**	< 0.01
Blood flow velocity ($cm.s^{-1}$)	21.52	30.23	NS

VO_{2SL} - symptom-limited oxygen uptake; VO_{2AT} - oxygen uptake at anaerobic threshold; W_{max} - maximal workload; HR_{max} - maximal heart rate.

- Regarding the relationship between the increase in VO_{2SL} and the increase in maximal heart rate (HR_{max}), a significant relationship was found in the bicycle group ($r = 0.64$; $P < 0.05$) but not in the ES group. A similar relationship was found between VO_{2SL} and the increase in exercise duration ($r = 0.68$; $P < 0.02$), and between VO_{2SL} and the increase in maximal workload ($r = 0.65$; $P < 0.05$), but in the bicycle group only.
- Doppler study of the common femoral artery showed an improvement of vasodilative capacities (i.e. the difference between resting and post-exercise velocities) after rehabilitation, but statistical significance was reached only in the ES group (*Tables 1, 2 and 3*).

Table 2
Values of parameters in the "ES" group before and after 5 weeks of low-frequency electrical muscle stimulation.

ES group (group 2)	**pre**	**post**	***P* value**
VO_{2SL} ($ml.kg^{-1}.min^{-1}$)	16.69	***17.67**	< 0.05
VO_{2AT} ($ml.kg.^{-1}min^{-1}$)	10.93	12.05	NS
W_{max} (W)	90.83	***98.33**	< 0.04
6-min walking test (m)	408.64	***480.18**	< 0.001
Exercise duration (s)	516	547	NS
HR_{max}($beats.min^{-1}$)	148	152	NS
Blood flow velocity ($cm.s^{-1}$)	29.09	***41.25**	< 0.01

VO_{2SL} - symptom-limited oxygen uptake; VO_{2AT} - oxygen uptake at anaerobic threshold; W_{max} - maximal workload; HR_{max} - maximal heart rate.

Table 3
Comparison of all the parameters in both groups after 5 weeks of given type of rehabilitation.

	Bicycle post	**ES post**	***P* value**
VO_{2SL} ($ml.kg^{-1}.min^{-1}$)	18.83	17.67	NS
VO_{2AT} ($ml.kg^{-1}.min^{-1}$)	***13.51**	12.05	< 0.001
W_{max} (W)	109.7	98.33	NS
6-min walking test (m)	***497.09**	480.18	< 0.01
Exercise duration (s)	627	547	NS
HR_{max} ($beats.min^{-1}$)	154	152	NS
Blood flow velocity ($cm.s^{-1}$)	30.23	***41.25**	< 0.01

VO_{2SL} - symptom-limited oxygen uptake; VO_{2AT} - oxygen uptake at anaerobic threshold; W_{max} - maximal workload; HR_{max} - maximal heart rate.

DISCUSSION

The global hypo-perfusion and chronic hypoxia in CHF induces gradual damage in functional and metabolic integrity of strength muscle mass. Consequent massive production of variety of pro-inflammatory cytokines stimulates apoptotic pathways leading to fibers atrophy (*11*), loss of strength, reduction of total muscle mass, global over-expression of anaerobic white fibers (fast glykolytic), and the development of general cachexia (*12, 13*). Electrical stimulation of strength muscles in humans has been shown to be valuable therapeutic intervention in neurology (*14, 15*), in post-surgery treatment and in the cases of long-term immobilization (*16-18*). However, the number of studies concerning the effects of LFES in cardiovascular rehabilitation is still very limited; our trial belongs to the few clinical reports that have focused on the therapeutic potential of low-frequency electrical stimulation in chronic heart failure. Recently, Harris et al. (2004) and Nuhr et al. (2004) published the results of first randomized trials comparing the home-based low-frequency ES training and classical exercise training; the results demonstrated that both methods could significantly influence the muscle strength, improve functional parameters including VO_{2SL} and VO_{2AT}, and improve also the quality of life in patients with CHF (*19, 20*). These results are very similar to those observed in our present study; 5 weeks of ES or bicycle training led to significant increase of VO_{2SL}, Wmax, distance walked in 6 minutes, and of HRmax in both groups. Also the exercise duration and VO_{2AT} were increased in both groups but the statistical significance was present only in the bicycle group. According to the Fick principle, VO_2 is the product of cardiac output by O_2 arterio-venous (AV) difference. Consequently, VO_2 = stroke volume x heart rate x AV difference. In the bicycle group, but not in the ES group, the improvement in VO_{2SL} seems to be mediated predominantly through the improvement in maximal heart rate, which itself appears to be the result of improved maximal workload and exercise duration. Such

a relationship was not found in the ES group, suggesting that electrical stimulation could act through a different mechanism. It is well known that exercise training induces a significant improvement of endothelial functions in patients with CHF (*21, 22*), and the contractions initiated by local electrical stimulation of the strength muscle may cause similar (or identical) vascular reactions as seen during physical exercises, especially exercise-induced reactive hyperemia in working muscles (*23, 24*). An acute rise of blood volume increases the shear stress on the vessel wall, which promotes the NO production and liberation (*25-27*). It is possible to suppose that stimulation-induced changes of blood flow velocity are most probably related to the modification of endothelial functions by long-term electrical stimulation, and thus may be NO-dependent. The significant increase of the blood flow velocity in femoral artery during stimulation observed in our study may reflect the importance of achieved global vascular benefit for the peripheral muscle mass after 5 weeks of ES. Although (from the point of view of effectiveness) it seems that both types of rehabilitation could positively influence the functional capacity and increase the resistance to fatigue, it is necessary to point out the existing differences between bicycle training and electrical stimulation. Electrical stimulation concerns only a part of the skeletal muscle mass and its activity is rather local, whereas exercise training on bicycle challenges the entire body. During the periods of stimulation we did not encounter any harmful effects related to the ES application, such as sudden blood pressure or heart rate changes; there were no complaints on muscular pain or skin burn (under the electrodes). Although our results are encouraging we take in account the existing limitations of the trial, first of all the limited number of patients included in the study. From clinical point of view the most important conclusion resulting from our study is that a significant improvement of functional capacity could be done either by ES or by conventional bicycle training. Next investigations should bring more detailed data, especially about possible interactions between the central and peripheral hemodynamic parameters during ES application. It is suggested that clinical trials on larger groups of patients are needed before the full introduction of ES in cardiovascular rehabilitation.

CONCLUSIONS

This study showed that:

- Improvement of exercise capacities can be achieved either by classical bicycle training or by electrical stimulation.
- Bicycle training improves more VO2SL, whereas electrical stimulation is more effective on sub-maximal exercise capacities.
- In the bicycle group, improvement of VO2SL seems to be mainly obtained through the increase in maximal heart rate, and probably through an improvement of muscle strength.
- Electrical stimulation seems to be more effective on the improvement of vasomotion.

Therefore, these 2 methods may prove to be complementary and could be used in combination, possibly with better results, especially in very sick patients.

Acknowledgement

This study was supported by the grant of Czech Ministry of Health IGA MZČR, No. NR 7983-3, 2004.

Abbreviations used

AV – arterio-venous, CHF – chronic heart failure, ES – electrical stimulation, HR_{max} – maximal heart rate, NYHA – New York Heart Association, VO_{2SL} – symptom-limited oxygen uptake, VO_{2AT} – oxygen uptake at anaerobic threshold, W_{max} – maximal workload

REFERENCES

1. Böhm M, Kilter H, Kindermann M. Mechanisms contributing to the progression of left ventricular dysfunction to end-stage heart failure. Eur Heart J 2003; 5(Suppl.): 114-21.
2. Nakamura M. Peripheral vascular remodeling in chronic heart failure: Clinical relevance and new conceptualization of its mechanisms. J Cardiac Fail 1999; 5: 127-38.
3. Suzuki K, Omiya K, Yamada S et al. Relations between strength and endurance of leg skeletal muscle and cardiopulmonary exercise testing parameters in patients with chronic heart failure. J Cardiol 2004;43(2):59-68.
4. Senden PJ, Sabelis LW, Zonderland ML et al. Determinants of maximal exercise performance in chronic heart failure. Eur J Cardiovasc Prev Rehabil 2004 Feb; 11(1): 41-7.
5. Coats AJ, Adamopoulos S, Radaelli A et al. Controlled trial of physical training in chronic heart failure. Exercise performance, hemodynamics, ventilation, and autonomic function. Circulation 1992; 85(6): 2119-31.
6. Adamopoulos S, Parissis J, Kroupis C et al. Physical training reduces peripheral markers of inflammation in patients with chronic heart failure. Eur Heart J 2001; 22(9): 791-7.
7. Schulze PC, Gielen S, Schuler G, Hambrecht R. Chronic heart failure and skeletal muscle catabolism: effects of exercise training. Int J Cardiol 2002;85(1):141-149.
8. Pette D, Staron RS. Mammalian skeletal muscle fiber type transitions. Int Rev Cytol 1997; 170: 143-223.
9. Maillefert JF, Eicher JC, Walker P et al. Effects of low-frequency electrical stimulation of quadriceps and calf muscles in patients with chronic heart failure. J Cardiopulm Rehabil 1998; 18: 277-82.
10. Vaquero AF, Chicharro JL, Gil L et al. Effects of muscle electrical stimulation on peak VO2 in cardiac transplant patients. Int J Sports Med 1998; 19(5): 317-22
11. Leri A, Claudio PP, Li Q et al. Stretch-mediated release of angiotensin II induces myocyte apoptosis by activating p53 that enhances the local rennin-angiotensin system and decreases the Bcl-2-to-Bax protein ratio in the cell. J Clin Invest 1998; 101: 1326-42.
12. Anker SD, Rauchhaus M. Heart failure as a metabolic problem. Eur J Heart Fail 1999; 1(2): 127-131.
13. Koller-Strametz J, Pacher R, Frey B, Kos T, Woloszczuk W, Stanek B. Circulating

tumor necrosis factor-alpha levels in chronic heart failure: relation to its soluble receptor II, interleukin-6, and neurohumoral variables. J Heart Lung Transplant 1998; 17(4): 356-62.
14. Jacobs PL, Nash MS. Modes, benefits, and risks of voluntary and electrically induced exercise in persons with spinal cord injury. J Spinal Cord Med 2001; 24(1): 10-8.
15. Hillegass EA, Dudley GA. Surface electrical stimulation of skeletal muscle after spinal cord injury. Spinal Cord 1999; 37(4): 251-7.
16. Gibson JN, Morrison WL, Scrimgeour CM, Smith K, Stoward PJ, Rennie MJ. Effects of therapeutic percutaneous electrical stimulation of atrophic human quadriceps on muscle composition, protein synthesis and contractile properties. Eur J Clin Invest 1989; 19(2): 206-12.
17. Vinge O, Edvardsen L, Jensen F, Jensen FG, Wernerman J, Kehlet H. Effect of transcutaneous electrical stimulation on postoperative muscle mass and protein synthesis. Br J Surg 1996; 83: 360-63.
18. Lewek M, Stevens J, Snyder-Mackler L. The use of electrical stimulation to increase quadriceps femoris muscle force in an elderly patient following a total knee arthroplasty. Phys Ther 2001; 81(9): 1565-71.
19. Harris S, LeMaitre JP, Mackenzie G, Fox KA, Denvir MA. A randomised study of home-based electrical stimulation of the legs and conventional bicycle exercise training for patients with chronic heart failure. Eur Heart J 2003; 24(9): 871-8
20. Nuhr MJ, Pette D, Berger R et al. Beneficial effects of chronic low-frequency stimulation of thigh muscles in patients with advanced chronic heart failure. Eur Heart J 2004 ; 25(2): 136-43.
21. Hambrecht R, Fiehn E, Weigl C, Gielen S, Hamann C, Kaiser R, Yu J, Adams V, Niebauer J, Schuler G. Regular physical exercise corrects endothelial dysfunction and improves exercise capacity in patients with chronic heart failure. Circulation 1998;98(24):2709-15.
22. Linke A, Schoene N, Gielen S, Hofer J, Erbs S, Schuler G, et al. Endothelial dysfunction in patients with chronic heart failure: Systemic effects of lower-limb exercise training. J Am Coll Cardiol 2001; 37: 392 - 397.
23. Duffy SJ, New G, Tran BT, Harper RW, Meredith IT. Relative contribution of vasodilator prostanoids and NO to metabolic vasodilation in the human forearm. Am J Physiol 1999;276(2):663-70.
24. Maiorana A, O'Driscoll G, Dembo L, Cheetham C, Goodman C, Taylor R, Green D. Effect of aerobic and resistance exercise training on vascular function in heart failure. Am J Physiol Heart Circ Physiol 2000;279(4):H1999-2005.
25. Sparks HV Jr, Belloni FL: The peripheral circulation:local regulation. Ann Rev Physiol 1978;40:67-92.
26. Fukai T, Siegfried MR, Ushio-Fukai M, Cheng Y, Kojda G, Harrison DG. Regulation of the vascular extracellular superoxide dismutase by nitric oxide and exercise training. J Clin Invest 2000;105(11):1631-9.
27. Kojda G, Cheng YC, Burchfield J, Harrison DG. Dysfunctional regulation of endothelial nitric oxide synthase (eNOS) expression in response to exercise in mice lacking one eNOS gene. Circulation 2001;103(23):2839-44.

FUNCTIONAL INDEPENDENCE MEASURE IN PATIENTS WITH STROKE

Pazdírek J., Nováková M., Šrubařová S.*, Svobodová J.*, Tarasová M.*, Bártlová B.*, Pospíšil P.*, Konečný L.*, Pospíšilová E.*, Vohlídalová I.*, Sosíková M.*, Siegelová J.*

Chronic Rehabilitation unit and *Department of Functional Diagnostics and Rehabilitation, St. Anna Teaching Hospital, Masaryk University, Brno, CZ

INTRODUCTION

The Chronic Rehabilitation unit of st. Anna Faculty Hospital in Brno, Masaryk University, is a facility focused on long term rehabilitation care. The unit and our team are dedicated to consecutive therapy of elder patients after strokes, operations, bone injuries and internal diseases. Every day we must solve a problem of adequate physical load in rehabilitation exercises with very old and seriously ill persons.

Standards of stage of functional disability of our patients may be very different. In contrast to standard of a young sportsman physical ability may be his or her ability to reach quickly the finish line - this determinates his placement on the winners steps. Patients with the age of 70 years and more have are quite different physical stage - in most cases their ability limited and they can reach only the small distance by waking and this condition does not allow their placement at home (1,2).

A correct functional abilities measurement and objectification of our non-pharmacologic therapeutic results are a main condition of contemporary evidence-based medicine. The difficulty of precise measurement in rehabilitation medicine is a result of wide diagnostic and biologic variability of our patients.

The problem of a uniform assessment of rehabilitation therapy outcomes was solved in USA in the middle of the 80s. With support from the U.S. Department of Education, National Institute on Disability and Rehabilitation Research, the Uniform Data System for Medical Rehabilitation (UDSMR) was developed as a method to document the severity of patient disability and the outcomes of medical rehabilitation. The national task force that created the Uniform Data Set for Medical Rehabilitation, started in 1983 and today, over 1,400 facilities in the United States, Canada, Hong Kong, Finland, the United Kingdom, and Australia subscribe to UDSMR, and the databases include over 2,500,000 patient records. UDSMR has territorial agreements with Hong Kong, Israel, Italy, Finland and South Africa (34,5).

A major part of UDSMR is Functional independence measure system (FIM) which evaluates the efficiency and effectiveness of rehabilitation program and can be used for standardization of impairment and level of disability. It can monitor patient functional gains and the results of the rehabilitation process and determine the severity of patient disability with the FIM™ instrument. This system generates data useful for uniform communication with families, care providers, insurance companies and is used for Inpatient rehabilitation facility prospective payment system services.

METHODS

Since the real FIM instrument is not yet available in our region, the hypothesis that the same methodology can be useful in our conditions for evaluation of therapeutic outcomes in long term rehabilitation therapy, was tested in our study.

The wide-spread use of the evaluation of patient functional abilities by a method derived from functional impairment measure (FIM) testing started at the beginning of year 2000. The number of cases tested by this method increased depending on the number of physiotherapists working in our department and from the middle of year 2002 all our patients that are placed in a rehabilitation program are evaluated by this method (3,4,5).

Considering the fact that the original FIM instrument is still not available here, in our methodology that is derived from it, we used all accessible informations from public sources, mainly from the Web, and we attempted to get as much written information about the original model as possible.

The FIM testing includes six main fields:
1.Self-Care, 2.Sphincter Control, 3.Transfers, 4.Locomotion - this four items form a motor subtotal score ranging from 13 to 91 points; 5.Communication and 6.Social cognition item form a Cognitive subtotal score ranging from 5 to 35 points. Total score is from 18 for totally dependent persons to 126 for completely independent persons.

Altogether eighteen items are evaluated and each of them has a seven point scale:

7 Complete Independence, 6 Modified Independence, 5 Supervision or Setup, 4 Minimal Contact Assistance, 3 Moderate assistance, 2 Maximal Assistance, 1 Total Assistance.

We took the interpretation for assessment of separate levels from a Slovakian authors (6,7) and the form we used is a translation of a form acquired from original reports 3,4,5).

The assessment was pursued by our physiotherapists who were all instructed by a rehabilitation medicine, specialized and obtained a special training in methodology.

From the total diagnostic range of patients treated in our unit we initially experimentally processed our largest homogenous set of patients after stroke.

RESULTS

In years 2002 and 2003, the total number of finished and administratively processed hospitalizations was 1,660. From this number, 379 patients were with diagnosis I 60 - I 69 general damage of central nervous system from vascular disorders with impairment of motor functions.

From all the patients, 159 (42%) were dismissed home, 133 (35%) were dismissed to social care facilities, 64 (17%) died, 23 patients (6%) returned to acute care departments due to complications.

In our subgroup of patients with stroke which was studied. The input and output FIM was evaluated for 119 out of 159 patients dismissed at home (75%), input and output FIM was evaluated for 90 out of 133 patients dismissed to social care facilities (68%).

Input and output FIM was elaborated in 205 cases from overall number of 379 patients after stroke who finished our rehabilitation program and were dismissed home or to social care facility.

All other entries are related to this subgroup of 205 patients who finished our rehabilitation program and have had correctly passed input and output FIM test.

Average and median age of all this patients was 77 years. Average and median age of patients dismissed home was 75 years. Average and median age of patients dismissed to

social care facilities was 79 years. The age distribution of our subgroup composed of 205 patients with stoke is presented in the Fig.1.

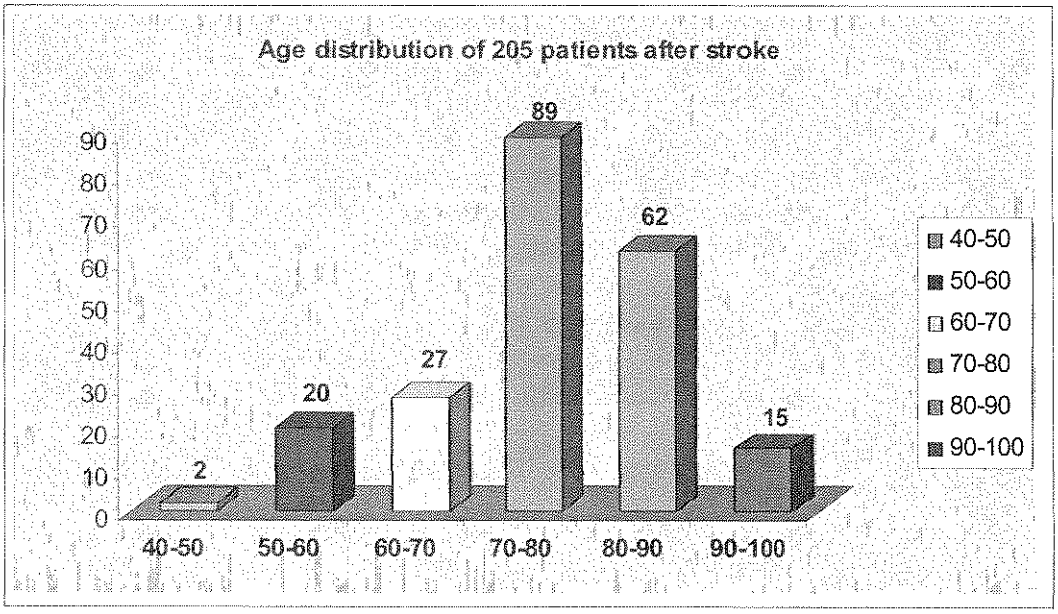

Fig.1

Average duration of rehabilitation was 44 days, Fig.2 summarize the duration of our patients. Average intensity of rehabilitation was 1 hour of exercises daily, five days a week.

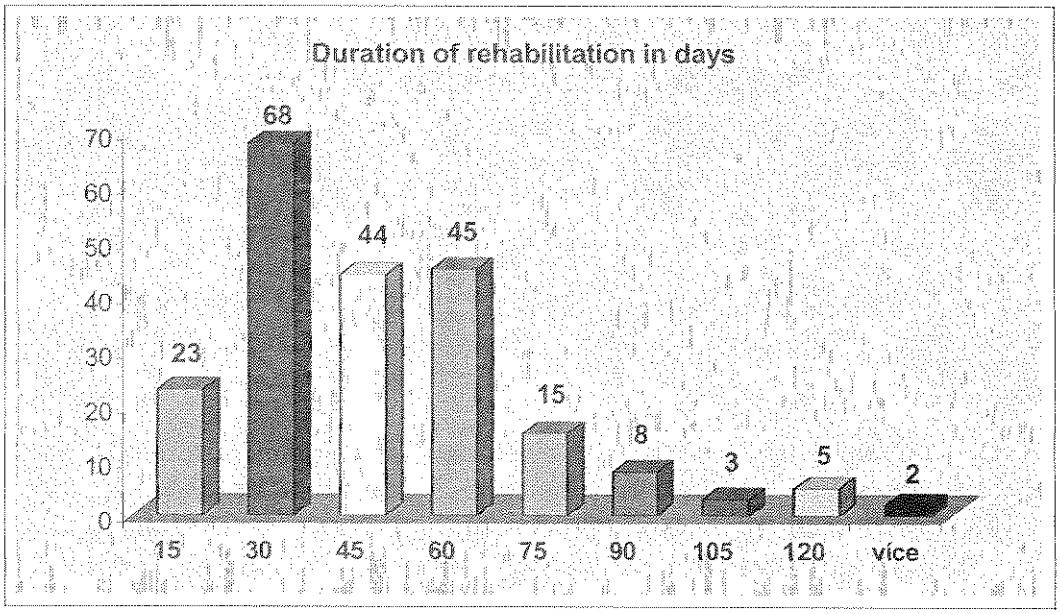

Fig.2

We compared values of motor (M) and cognitive (C) score in admission and before dismissed from our unit separately for all 205 patients, then we dividend for a set of 118 patients dismissed home and for a set of 88 patients dismissed to social care facilities.

For statistical evaluation of differences between motor (M) and cognitive (C) scores of patients at the time of admission and dismissed from our unit, a nonparametric Wilcoxon pair test was used. This test proved statistically highly relevant score improvement on dismissed in both parameters ($P<0.0001$). Identical result - in all tested sets - relevant score improvement on dismissed was obtained by testing with parametric Student t-test. The identical results obtained by two statistical methods clearly prove reliability of obtained results. In data of both parameters of motor (M) and cognitive (C) score we can observe an improvement trend, documented by Wilcoxon test relevance.

Comparison of median FIM score in admission and before dismiss is in the Fig.3

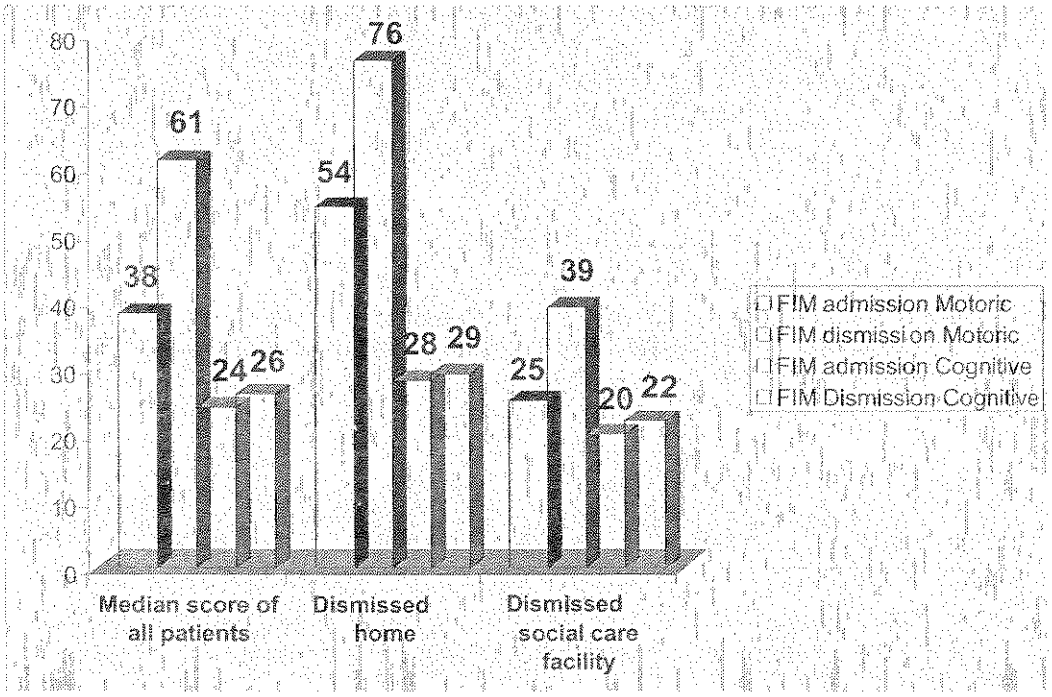

Fig.3

DISCUSSION

During the treatment of 205 patients with stroke at our department a highly relevant improvement in Functional independence measure score tested by FIM method was reached. We consider this functional improvement a result of implementation of rehabilitation therapy for motor score. The improvement in cognitive score reflects a suspicious improvement of general health conditions of treated persons because no specific cognitive training was performed. Formerly widely accepted view, that rehabilitation of patients over 70 year of age is purposeless is evidently obsolete and we are convinced, that this fact must be taken into consideration in all facilities of consecutive care where rehabilitation therapy is still unsatisfying (8,9,10,11,12,15). The improvement of motor

and cognitive state of seriously ill patients enable their dismiss home or relieve the burden of care for nursing staff in social care facilities.

The FIM testing method may be useful for evaluation of long term rehabilitation effect also in conditions of healthcare system in Czech Republic (16).

REFERENCES

1. Dufek M. Cerebrovaskulární onemocnění ve stáří, Neurologie pro praxi 2003, 1, p. 14-20.
2. Fasoli S.E, Krebs H.I, Ferraro M.,Hogan N., Volpe T.: Does shorter rehabilitation limit potential recovery poststroke? Neurorehabilitation and neural repair 18(2) 2004 p.88-94.
3. Functional independence measure(FIM) http://www.sci-queri.research.med.va.gov/fim.htm
4. FIM instrument http://www.jasonprogram.org/Articles/fim.pdf
5. Jette A.M., Haley S.M, Pengsheng Ni.:Comparison of Functional Status Tool Used in Post-Acutre Care - Health Care Financing Review, Spring 2003,24.(3), p.13-24.
6. Malý M.: Poranenie miechy a rehabilitácia. Bratislava: Bonus, 1999. 577 p
7. Malý M.:Testovanie funkčnej sebestačnosti. Rehabilitácia, 2, 2001, p 69-75.
8. Quality of life measurement in rehabilitation: Misleading evidence. Leuven- May 8th 2004, http://www.uzleuven.be/UZRoot/files/webeditor/Leuven_08-05-04TESIO.pdf
9. Society Compendium Document A4 (1997) Evidence of effectiveness to assist purchasing of general rehabilitation, and specific packages for rehabilitation of stroke and fractured neck of femur
10. Švestková O. Patofyziologický rozbor funkční zdatnosti organizmu, Autoreferát doktorandské práce, Univerzita Karlova Praha 2003, 30 p
11. Švestková O. Možnosti posouzení funkčních schopností,aktivit a participací, Autoreferát doktorandské práce, Univerzita Karlova, Praha 2004, 43 p.
12. Uniform data systém for medical rehabilitation homepage - http://www.udsmr.org/
13. Vaňásková E., Tošnerová V.: Náhlé cévní příhody mozkové - testování v rehabilitační péči, Rehabilitace a fyzik. lékařství, 1994,1, p 28-31
14. Vaňásková E.: Rehabilitace nemocných po cévní mozkové příhodě na neurologickém oddělení, Čes.a Slov. Neurol.Neurochir., 59/92,1996,NoI, p 33-39.
15. Vaňásková E.: Hodnocení nemocných po cévní mozkové příhodě testy soběstačnosti na lůžkovém rehabilitačním pracovišti, Rehabilitace a fyz.lékařství, 2003, 10 (2), p. 60-64 .
16. Věstník ministerstva zdravotnictví České republiky částka 9 vydáno: září 1998 Metodická opatření 12-koncepce následné lůžkové péče v ČR.

CONTROL OF BLOOD PRESSURE IN PATIENTS WITH DIABETES MELLITUS AND ESSENTIAL HYPERTENSION

Svačinová H., Siegelová J., Fišer B., Dušek J., Jančík J., Ošmerová J., Tarasová M., Chludilová V., Pospíšil P., Konečný L., Svoboda L.

Department of Functional Diagnostics and Rehabilitation
St. Anna´s Teaching Hospital, Faculty of Medicine, Masaryk University, Brno

INTRODUCTION

The coincidence of type 2 diabetes mellitus and hypertension is considered to be a malignant combination participating in additional increase of cardiovascular risk and mortality and they are clustered with other pathological condition (insulin resistance, abdominal obesity, dyslipidemia) in metabolic syndrome (1). Metabolic syndrome is accompanied with increased sympathetic activity that increases the cardiovascular risk (especially the risk of sudden death and life-threatening arrhythmias); therefore, the evaluation of cardiovascular autonomic functions is reasonable in these patients (1-5). Determination of baroreflex sensitivity using non-invasive method of measuring - spectral analysis of systolic blood pressure (SBP) and cardiac intervals (CI) fluctuation can contribute to evaluation of cardiovascular risk and the effect of therapy (both non-pharmacological and pharmacotherapy). Depressed value of BRS indicates an increased sympathetic nervous activity. Our study was focused on the evaluation of potential favourable effect of walking training on the heart rate baroreflex sensitivity compared to effect of pharmacotherapy of hypertension in patients with type 2 diabetes mellitus and hypertension.

PATIENTS AND METHODS

In two groups of diabetics type 2 - with hypertension controlled by ACE inhibitors or Ca-channel-blockers (DMH, n = 7, age 56 ± 4 years) and without hypertension (DMN, n = 6, age 60 ± 8years). BRS was evaluated before (1) and after (2) 12 weeks of walking training program that consist of daily 30-60 min walking at least 3-4x a week. Furthermore, BRS was measured in patients with essential hypertension without treatment (EH, n =11, age 60 ± years), in normotensives (N, n = 11, age 58 ± 6years), in patients with essential hypertension and monotherapy of ACE inhibitors or Ca-channel blockers (EHT1, n = 12, age 59 ± 6 years) and in hypertensives with combined therapy of

trandolapril and dilthiazem (EHT2, n = 18, age 48 ± 5 years). BRS was determined by a 5-minute continuous beat-to-beat recording of blood pressure according to Peňáz method (6) (Finapres Ohmeda) at metronome controlled breathing frequency of 0,33 Hz. The BRS value was calculated by spectral analysis of spontaneous fluctuations of systolic blood pressure (SBP) and cardiac intervals (CI). The value of cross-spectral power density of CI and SBP fluctuation was divided by the value of power spectral density of systolic blood pressure fluctuation at 0,1Hz. The value obtained, i.e. modulus, was considered to be the measure of BRS. The value of this function at frequency of 0,1Hz corresponds to BRS (ms/mm Hg).

The experimental protocol was approved by the local Ethics committee, prior participation all subjects gave written informed consent.

Statistical analysis of data was performed using Wilcoxon paired test and ANOVA; The significant differences were considered at $p < 0,05$. The data are performed as mean ± SD and were processed by Microsoft Excel 97.

RESULTS

The results of BRS of all patients groups are given in *Table 1.*

The walking training program increased significantly BRS in patients with type 2 diabetes mellitus (both with and without hypertension). The BRS value in non-diabetic groups was significantly increased in hypertensive patients with combined medication **(EHT2)** and in normotensives **(N)** compared to hypertensive group without therapy **(EH)** and hypertensives with monotherapy **(EHT1)**. Systolic and diastolic blood pressure was significantly increased in hypertensive group without therapy **(EH)**.

Table 1. Results of BRS in examined patients groups.

Patients groups	**BRS** (ms/mmHg) (mean ± SD)	**SBP** (mm Hg) (mean ± SD)	**DBP** (mm Hg) (mean ± SD)	**CI** (ms) (mean ± SD)
DMN1	3,1 ± 1,1	119 ± 17	71 ± 10	775 ± 114
DMN2	4,7 ± 1,2*	122 ± 13	71 ± 13	788 ± 69
DMH1	5,1 ± 1,8	140 ± 26	70 ± 14	946 ± 146
DMH2	7,2 ± 2,3*	135 ± 18	68 ± 11	947 ± 170
EH	4,7 ± 1,8	156 ± 12‡	98 ± 7 ‡	759 ± 159
N	7,8 ± 3,8†	121 ± 10	75 ± 8	789 ± 115
EHT1	4,9 ± 2,7	129 ± 9	85 ± 7	750 ± 93
EHT2s	8,2 ± 3,4†	128 ± 8	82 ± 9	789 ± 60

Legend: DMN1, DMN2 : normotensive diabetics before(1) and after(2) walking training; DMH1, DMH2 : diabetics with hypertension before(1) and after(2) walking training; EH: hypertensives without therapy; N: normotensive patients ; EHT1: hypertensive patients with monotherapy; EHT2: hypertensive patients with combined therapy.
* statistically significant at $p< 0,05$ against to DMN1 a DMH1; † statistically significant at $p< 0,05$ against to EH, EHT1; ‡ statistically significant at $p < 0,05$ against to all groups

DISCUSSION

In patients with diabetes mellitus type 2 the average prevalence of hypertension is about 80%. In the majority of patients, diabetes type 2 is associated with insulin resistance (IR) that appears to be a key factor in the development of other pathological conditions (including hypertension), involved in the metabolic syndrome. Autonomic dysfunction with increased sympathetic activation expressed by low value of baroreflex sensitivity along with depressed heart rate variability (HRV) is characteristic feature of metabolic syndrome. Several studies have demonstrated that depressed BRS and HRV are strong independent risk factors for sudden cardiac death in patients after myocardial infarction (7, 8) and the authors have later demonstrated favourable impact of exercise on BRS and improvement of patients´ prognosis (9). Moreover, in diabetic patients decreased BRS and HRV can be considered to be an early sign of cardiovascular autonomic neuropathy (CAN) accounting for an approximately five fold increase in mortality and some studies have suggested exercise can improve diabetic cardiovascular autonomic dysfunction (10, 11, 12). One of the main therapeutic objectives in patients with diabetes type 2 is to decrease both IR and increased sympathetic tone represented by low value of baroreflex sensitivity. Physical activity is one of the non-pharmacological therapeutic means that favourably influenced insulin sensitivity (13) and BRS . In some studies the authors found out that ACE inhibitors are able favourably influenced not even blood pressure but insulin sensitivity and BRS (14, 15).

We conclude that the walking training program increased significantly BRS in patients with type 2 diabetes mellitus both with and without hypertension. Comparing this non-pharmacological treatment to pharmacotherapy of hypertension, we found out that the value of BRS in normotensives (**N**) corresponds with BRS value in the diabetics with hypertension after training (**DMH_2**) and hypertensives with combined medication (**EHT_2**).

The results support important role of regular physical activity in favourable affecting of cardiovascular autonomic function and non-pharmacological intervention of cardiovascular risk factors associated in metabolic syndrome.

Acknowledgement

This study was supported by a grant CEZ: J037/98: 141100004 from the Ministry of Education of the Czech Republic.

REFERENCES

1. Reaven GM, Lithell, H, Landsberg, L. Hypertension and associated metabolic abnormalities - the role of insulin resistance and the sympatoadrenal system. N. Eng. J. Med. 1996; 334: 364 - 381
2. Landsberg L. Insulin-mediated sympathetic stimulation: role in the pathogenesis of obesity-related hypertension (or, how insulin affects blood pressure, and why). Journal of Hypertension 2001; 19: 532 - 528
3. Mancia G. The sympathetic nervous system in human hypertension. J. Hypertens. 1997; 15: 1553 - 1565Peňáz J. Photoelectric measurements of blood pressure, volume and flow in the finger. Digest of 10th Internat. Conf. Med. Biol. Engeneering, Dresden, 1973; 104 s.

4. Grassi G. Role of the sympathetic nervous system in human hypertension. J. Hypertens. 1998; 16: 1979 - 1987
5. Pikujämsä SM, Huirikuri HV, Airaksinen KEJ et al. Heart rate variability and baroreflex sensitivity in hypertensive subjects with and without metabolic features of insulin resistance syndrome. American Journal of Hypertension 1998; 5: 523 - 531
6. Peňáz J. Photoelectric measurements of blood pressure, volume and flow in the finger. Digest of 10th Internat. Conf. Med. Biol. Engeneering, Dresden, 1973; 104 s.
7. LaRovere MT, Bigger JT, Marcus FI. et al. Baroreflex sensitivity and heart rate variability in prediction of total cardiac mortality after myocardial infarction. Lancet 1998; 351: 478 - 484
8. Semrád B, Honzíková N, Fišer B. Metody určení rizika náhlé srdeční smrti u nemocných po infarktu myokardu. Cor Vasa 1994: 36: 295 - 298
9. LaRovere MT, Bersano Ch, Gnemmi M. et al. Exercise inducted increase in baroreflex sensitivity predicts improved prognosis after myocardial Infarction. Circulation 2002; 106: 945 - 949
10. Howorka K, Pumprla, J, Haber, P et al. Effects of physical training on heart rate variability in diabetic patients with various degrees of cardiovascular autonomic neuropathy. Cardiovasc. Res. 1997; 34: 206 - 214
11. Svačinová H, Siegelová J, Olšovský J et al. Effect of exercise training on baroreflex sensitivity in patients with type 2 diabetes mellitus. J. Hypertension 2001; 19, Suppl. 2, s. S14
12. Frattola A, Parati G, Gamba P et al. Time and frequency domain estimates of spontaneous baroreflex sensitivity provide early detection of autonomic dysfunction in diabetes mellitus. Diabetologie 1997; 40: 1470 - 1475
13. Dela F, Ploug T, Handberg A et al. Physical training increases GLUT-4 protein and mRNA in patients with NIDDM. Diabetes, 1994; 43: 862 - 865
14. Siegelová J, Fišer B, Dušek J. Baroreflexní senzitivita srdeční frekvence u pacientů s esenciální hypertenzí po léčbě verapamilem a trandolaprilem. Cor Vasa 1998; 4 (suppl.): 12
15. Ylitalo A, Airaksinen KE, Sellin L et al. Effects of combination of therapy on baroreflex sensitivity and heart rate variability in systemic hypertension. Am. J. Cardiol. 1999; 83: 885 - 889

MUSCULAR STRENGTH IN PATIENTS WITH CHRONIC HEART FAILURE AFTER SOME WEEKS OF LOW-FREQUENCY ELECTRICAL STIMULATION

Mífková L.[1], Dobšák P.[1], Jančík J.[1], Eicher J.C.[2], Svačinová H.[1], Placheta Z.[1], Kožantová L.[1], Chludilová V.[1], Vohlídalová I.[1], Sosíková M.[1],Špinarová L.[3], Vítovec J.[3], Wolf J.E.[2], Siegelová J.[1]

[1] Department of Functional Diagnostics and Rehabilitation, St. Anna Faculty Hospital in Brno, Faculty of Medicine, Masaryk University, Brno, Czech Republic,
[2] Center of Cardiology II, Hôpital du Bocage Dijon, France
[3] I[st] Clinic of Cardioangiology, St. Anna Faculty Hospital in Brno, Faculty of Medicine, Masaryk University, Brno, Czech Republic

INTRODUCTION

The syndrome of chronic heart failure (CHF) is typically characterized by decreased exercise capacity with reduced peak oxygen consumption. The exercise abnormalities are closely related to impaired skeletal muscle behaviour. The skeletal muscle oxidative metabolism is depressed, intracellular pH levels decrease, phosphocreatine depletion during exercise increases and phosphocreatine resynthesis decreases (1). The increased sympathetic tone and stimulation of the renin-angiotensin-aldosterone system influence the redistribution of regional blood flow and create endothelial dysfunction of all vessels. This leads to an impaired peripheral vascular dilatation in response to vasodilator stimuli and reduction of blood flow and O_2 supply in skeletal muscles (2). Chronic hypoxia damages strongly the structural and metabolic integrity of muscle fibers. The resulting general atrophy decreases the power and fatigue resistance of muscles. Chronic low-frequency electrical stimulation (LFES) has been shown to decrease fatigue and to improve the performance of skeletal muscles.

The aim of this study was to investigate whether the long-termed LFES improves skeletal muscle performance and the rating of perceived exertion.

MATERIALS AND METHODS

PATIENTS

A group of 10 patients (age 54 ± 7 years, ejection fraction 18 ± 2 %) diagnosed with CHF, classified as NYHA grades III to IV, were included in the study. They all had undergone coronarography, were symptomatically stable and on optimal pharmacological treatment (ACEI, betablockers, diuretics) that remained unchanged throughout the study.

PROTOCOL OF LFMES APPLICATION

The muscles to be stimulated were quadriceps muscles. Special rectangular electrodes 80x100mm (St.Cloud International, Chantonnay, France) were positioned on the thighs cca 3cm under inguinal fold and 2cm over the upper patella border. Electrical stimulation was performed 1 hour/day, 7 days a week for 3 (5) weeks, using dual-channel stimulator Elpha 2000 (Danmeter, Odense, Denmark). The stimulator delivered a biphasic current of 10 Hz frequency. The pulse duration was 200 msec with an "on-off" mode of stimulus (20 s stimulation, 20 s pause). The maximal stimulation amplitude was 60 mA.

MEASUREMENTS

To determine the maximal muscle strength (F_{max}), an isometric dynamometry of quadriceps muscles was performed every week, using a PC-2 SDT dynamometer (Czech Republic).

To evaluate the influence of LFES on cardiovascular reactivity, two basic hemodynamic parameters were measured: blood pressure (systolic and diastolic - SBP and DBP) and heart rate (HR) before and after LFES application.

The rating of perceived exertion (RPE) was expressed using the Borg scale.

STATISTICAL ANALYSIS

The Wilcoxon paired test was used for statistical analysis. The results were expressed as mean ± SD values. A P value < 0.05 was considered as significant.

RESULTS

A significant improvement of the quadriceps muscle strength (*Table 1*) and of the RPE (*Table 2*) was observed after 3 and 5 weeks of stimulation (see the table below). SDB, DBP and HR values changes were not significant during all the period of stimulation.

Table 1

Results of muscle strength measurements before and after 3 and 5 weeks of low-frequency electrical stimulation in patients with chronic heart failure

Muscle strength	Before LFES ($\bar{x}$ ± SD)	After 3 weeks ($\bar{x}$ ± SD)	After 5 weeks ($\bar{x}$ ± SD)
F_{max} (N)	15.7 ±103.9	249.1 ±100.6 *	334.1 ± 111.9 **

F_{max}, maximal muscle strength; N, Newtons; LFES, low-frequency electrical stimulation; $\bar{x}$, mean; SD, standard deviation; *, $P < 0.05$; ** $P < 0.01$.

Table 2

The changes of the rating of perceived exertion before and after 3 and 5 weeks of low-frequency electrical stimulation in patients with chronic heart failure

RPE (Borg scale)	Before LFES ($\bar{x} \pm SD$)	After 3 weeks ($\bar{x} \pm SD$)	After 5 weeks ($\bar{x} \pm SD$)
Exertion	69 ± 25	64 ± 26	51 ±19
Dyspnea	21 ±16	17 ± 17 *	12 ± 8 *

RPE, rating of perceived exertion; $\bar{x}$, mean; SD, standard deviation; *, $P < 0.05$.

DISCUSSION

The results of this study demonstrated a significant positive impact of the LFES after 3 and 5 weeks of stimulation on the muscle performance of quadriceps muscles in patients with CHF. Analysis of RPE, SBP, DBP and HR showed that LFES was subjectively well tolerated and did not exhibit any harmful effect on hemodynamic parameters.

In 2002 we have published our first results together with French authors with low-frequency electrical stimulation of skeletal muscles in patients with chronic heart failure (3). The low-frequency stimulation was well tolerated by all subjects and after one week improved the quadriceps muscle strength by 28 %. The results of magnetic resonance imaging analysis of the gastrocnemius muscle showed a significant increase in muscle volume after stimulation.

In previous our study we have found that three weeks of LFES significantly increased both muscle strength and blood flow velocity. It was concluded that LFES may improve the structural and functional patterns of skeletal muscles and may be useful in the treatment of patients with severe chronic heart failure (4). The beneficial effects of chronic low-frequency stimulation of thigh muscles in patients with advanced chronic heart failure were described also by Nuhr et al (5) and Quittan et al (6).

Experiments with LFES confirmed the crucial stimulation-induced changes in skeletal muscles, leading to the transformation of fast fatigable muscles, toward slower, fatigue-resistant ones (7,8). Previous studies showed that LFMES increases capillary density and enhances perfusion in rat and rabbit strength muscles (9,10). The published results showed also a significant improvement of exercise capacity parameters (VO_{2peak}, VO_{2AT}) in patients with CHF after 5 weeks of LFMES (11).

The method of LFES can be used also in heart transplantation candidates with cardiac pacemakers (12).

A c n o w l e d g e m e n t

This work is supported by the grants IGA MZ ČR NR 7983-3 and MŠM:141100004.

REFERENCES

1. Wielenga RP, Coats JS, Mosterd W, Huisveld I. The role of exercise training in chronic heart failure. Heart 1997; 78: 431-36.
2. Hornig B, Maier V, Drexler H. Physical training improves endothelial function in patients with chronic heart failure. Circulation, 1996; 93:210-14.
3. Jančík J, Siegelová J, Homolka P et al. Low-frequency electrical stimulation of skeletal muscles in patients with chronic heart failure. Scripta medica, 2002;75(4):203-8.
4. Jančík J, Dobšák P, Eicher JC et al. Increase in muscle strength after low-frequency electrical stimulation in chronic heart failure. Scripta medica, 2003;76(5):285-90.
5. Nuhr MJ, Pete, Berger R et al. Beneficial effects of chronic low-frequency stimulation of thigh muscles in patients with advanced chronic heart failure. Eur Heart J 2004;25(2):104-106.
6. Quittan M, Sochor A, Wiesinger GF et al. Strength improvement of knee extensor muscles in patients with chronic heart failure by neuromuscular electrical stimulation. Artif Organs 1999;23(5):432-5.
7. Reichmann H, Hoppeler H, Matthieu-Costello O et al. Biochemical and ultrastructural changes of skeletal muscle mitochondria after chronic electrical stimulation in rabbits. Pflugers Arch 1985; 404: 1-9.
8. Termin A, Pette D. Changes in myosin heavy-chain isoform synthesis of chronically stimulated rat fast-twitch muscle. Eur J Biochem 1992; 204: 569-73.
9. Matthieu-Costello O, Agery PJ, Wu L et al. Capillary-to-fiber ratio in rat fast-twitch nindlimb muscle after chronic electrical stimulation. J Appl Physiol 1996; 80: 904-09.
10. Skorjanc D, Jaschinski F, Heine G, Pette D. Sequential increases in capillarization and mitochondria enzymes in low-frequency stimulated rabbit muscle. Am J Physiol 1998; 274: 810-18.
11. Maillefert JF, Eicher JC, Walker P et al. Effects of low-frequency electrical stimulation of quadriceps and calf muscles in patients with chronic heart failure. J Cardiopulm Rehabil 1998;18:277-82.
12. Wiesinger FG, Crevenna R, Nuhr MJ et al. Neuromuscular electric stimulation in heart transplantation candidates with cardiac pacemakers. Arch Phys Med Rehabil 2001;82(10):1476-7.

RELATIONSHIP BETWEEN INTIMA MEDIA THICKNESS AND BAROREFLEX SENSITIVITY IN ESSENTIAL HYPERTENSION

Siegelová J, Hofírek I*, Fišer B, Jančík J, Vank P, Svačinová H, Dušek J, Konečný L, Pospíšil P, Ošmerová J, Tarasová M, Chludilová V,Vohlídalová I., Sosíková M. Svoboda L, Placheta Z.

Dept. of Functional Diagnostic and Rehabilitation
*II Dept. of Internal Medicine, St.Anna`s Teaching Hospital, Faculty of Medicine, Masaryk University in Brno, Czech Republic

INTRODUCTION

The increased diastolic and systolic blood pressure is an important risk factor of stroke and coronary heart disease (1). In elderly patients we observe more frequently the isolated systolic hypertension because naturally the diastolic pressure decreases after the age of 50 years (2). It is evident that the increase pulse pressure (the difference between systolic and diastolic pressure, PP) is seen in patients with isolated systolic hypertension. The importance of increased pulse pressure as a risk factor for mortality and morbidity was reviewed (3). It is also well known that decreased baroreflex sensitivity is a risk factor for mortality in ischemic heart disease patients (4). Recently we observed lower baroreflex sensitivity in patients with higher pulse pressure (5). The question arises if the decrease of baroreflex sensitivity can be caused by lower distensibility of carotid arteries. For this reason we analysed of the relationship between pulse pressure and intima -media thickness (IMT) in patients with essential hypertension treated with ACE inhibitors or Ca-antagonists. The relationship between pulse pressure and baroreflex sensitivity in these patients was the second aim of the present study.

MATERIAL AND METHODS

We examined 30 patients (all men) with essential hypertension treated with ACE inhibitors or Ca-antagonists. The patients were divided in two subgroups using the pulse pressure (PP) according to mean blood pressure (MAP) diagram as is shown in Fig.1. The division was done according to the regression line expressing the linear relationship between MAP and PP. The dots up and right of the line corresponded to the patients with higher PP (group HPP), the dots under and right from the line corresponded to the patient with low PP (group LPP). The mean blood pressure MAP was similar in both groups.

We measured IMT of both common carotid arteries by Doppler echocardiography (SONOS 5500, Hewlet Packard, USA).

Baroreflex sensitivity was determined using two methods. Baroreflex sensitivity of heart rate (BRS) in ms/mmHg was measured by spectral method. Systolic (SBP) and diastolic blood pressure (DBP) were noninvasively continuously recorded beat-to-beat (Finapres, Ohmeda USA) for 5 minutes during metronome controlled breathing 0.33 Hz.

Power spectra of cardiac interval variations (Fci*ci) and cross-spectra between SBP and cardiac intervals (Fci/sbp) were calculated, the modulus between Fci*ci and Fci/sbp at 0.1 Hz corresponds to BRS (BRS=Fci*ci/(Fci/sbp)). Only calculations where coherence calculated from Fci, power spectra of systolic blood pressure (Fsbp) and Fci/sbp at 0.1 Hz was higher than 0.5 were taken into account.

Blood pressure component of the baroreflex was measured by following method. Two inflatable cuffs were placed on both thighs of supine patient. The pressure in the cuffs was abruptly increased on suprasystolic value a kept constant for 5 minutes to induced ischemia in both lower extremities. Then the cuff pressure was rapidly released. A decrease of peripheral resistance in lower extremities resulted in blood pressure immediately decrease 10-30 mmHg, lasted some time and than returned back to the original level under the influence of baroreflex. The method was described elsewhere (6). The speed of the SBP and DBP return to the original level was evaluated. The curve of SBP and DBP return is of sigmoid shape, maximum slope of the curve expressed in mmHg/s for both SBP (SBP mmHg/s) and DBP (DBP mmHg/s) corresponds to the magnitude of blood pressure component of the baroreflex.

The results are summarizes as means ± SD. The statistical significance of differences between both groups was determined by Wilcoxon non-parametric test.

The Local Ethics Committee of Teaching Hospital approved the study and all patients signed informed consent.

RESULTS

The results are seen in the Table 1 and Table 2. Our results show that the treated hypertensives with higher pulse pressure are older, have a lower gain of the baroreflex and have a larger IMT.

Fig. 1

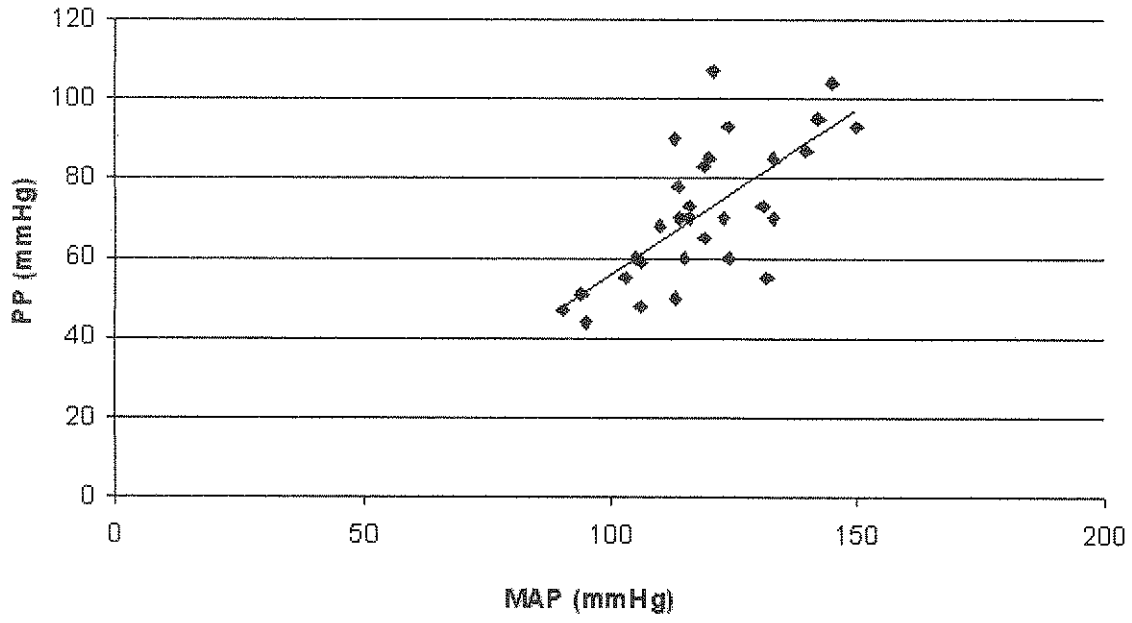

Table 1
Age, systolic (SBP) and diastolic blood pressure (DBP), pulse pressure (PP) and mean arterial pressure (MAP) in hypertensives with low (PPL) and high pulse pressure (PPH). The results are given in the table (mean SD):

Age	**SBP**	**DBP**	**PP**	**MAP**
Years	mmHg	mmHg	mmHg	mmHg
PPL 57 9	135 21	75 11	62 14	118 16
PPH 65 9	*146 18	66 11	81 15*	119 13

($p<0.05$: * set PPL versus PPH.; Wilcoxon)

Legend: SBP – systolic blood pressure, DBP – diastolic blood pressure, PP pulse pressure, MAP – mean arterial pressure

Table 2
Intima -media thikness (IMT), baroreflex sensitivity (BRS), pulse component of baroreflex for SBP, DBP in hypertensives with low (PPL) and high pulse pressure (PPH). The results are given in the table (mean SD):

ITH	**BRS**	**pulse component of baroreflex**	
mm	ms/mmHg	SBPmmHg/s	DBPmmHg/s
PPL 0.71±0.12	3.73 +3.06	1.05+0.78*	0.71+0.53
PPH 0.94±0.24*	2.14+2.27	0.71+0.50	0.59+0.45

($p<0.05$: * set PPL versus PPH.; Wilcoxon)
The results are given in the table (mean SD):

Legend: ITH -intima media thickness, BRS baroreflex sensitivity, pulse component of baroreflex for SBP, DBP

DISCUSSION

In a recent study we observed that PP magnitude is not important factor in regard to peripheral resistance of cerebral arteries (7). However in the present study we found an increased IMT in increased PP by equal MBP. To answer the question what is the cause and what is the consequency is not simple. Increased IMT corresponds to the higher stiffness of carotid arteries. Increased stiffness of aorta is the reason for increased pulse pressure and increased blood pressure is a stimulus increasing the stiffness of the arteries. It can be speculate that the increased stiffness is the culprit of the decreased baroreflex sensitivity. Baroreceptors are stretch receptors in the carotid sinus. Lower distensibility of a.carotis because of higher stiffness can be the cause of decreased baroreflex sensitivity. The decrease of baroreflex sensitivity in patients with hypertension

was seen in several studies (8, 9). Also the decline of baroreflex sensitivity with age was observed (10). This fact is in accord with our finding of higher age in our HPP group.

Icreased IMT has been shown to be independent predictor of adverse cardiovascular events (11). The results of study of the relationship between IMT and BRS is contraversial. It was shown that BRS correlates with ITM in the carotic sinus area and not in the common carotid region (12).

We didn't observe the significantly lower baroreflex sensitivity of all parameters, only in SBPmm/Hg. This can be explained by large variability of baroreflex sensitivity, like the variability of blood pressure which is also not constant at repeated measurement. In our results we can see that also BRS and DBPmm/Hg is lower in the HPP group, only the statistical significance was not reached.

Our results are not without clinical significance. Low baroreflex sensitivity only insufficiently suppress the increase of sympathetic activity, the important factor participated in sudden cardiac death. It is possible that low baroreflex can contribute to the increased risk for mortality in patients with high pulse pressure.

Acknowledgement
This study was supported by grant CEZ: MSM 141100004.

REFERENCES

1. MacMahon S, Peto R, Cutle J et al. Blood pressure, stroke and coronary heart disease, part 1: prolonged differences in blood pressure. Lance 1990; 335:765-774.
2. Burt VL, Whelton P, Roccella EJ et al. Prevalence of hypertension in the US adult population. Results from the Tird National Health and Nutrition Examination Surgery 1988-1991. Hypertension 1995; 25:305-313.
3. Dart AM, Kingwell BA.Pulse pressure - a review of mechanisms and clinical relevance. J Am Coll Cardiol 2001; 37:975-984.
4. LaRovere MT, Bigger, JT, Marcus et al. Baroreflex sensitivity and heart rate variability in prediction of total cardiac mortality after myocardial infarction. Lancet 1998; 351:478-484.
5. Siegelová J, Hofírek I, Fišer B et al. Relationship between pulse pressure and baroreflex sensitivity in patients with essential hypertension. Scripta medica 2002; 75; 5:101-104.
6. Savin E, Siegelová J, Fišer B, Bonnin P. Intra- and extracranial artery blood velocity during a sudden blood pressure decrease in humans. Eur J Appl Physiol 1997; 76:289-93.
7. Siegelová J, Hofírek I, Fišer B, et al. Pulse pressure and carotid blood flow in essential hypertension. Scripta medica 2003; 76; 5:301-304.
8. Grassi G, Gattaneo BM, Seravalle G, Lanfranchi A, Mancia G. Baroreflex control of sympathetic nerve activity in essential and secondary hypertension. Hypertension 1998; 31:68-72.
9. Siegelová J, Fišer B, Dušek J, AL-Kubati M: Baroreflex-Senstivitaetsmessung bei Patienten mit essentieler Hypertonie: Einfluss von Enalapril. Nieren und Hochdruckkrankheiten 1995; 24:20-22.
10. Gribbin B, Pickering EG, Sleight P, Peto R. Effect of age and high blood pressure on baroreflex sensitivity in man. Circ Res 1971; 29:424-431.

11. Scuteri A, Najjar SS, Muller DC et al. Metabolic Syndrome amplifies the age-associated increases in vasular thickness and stiffness. J American College of Cardiology 2004; 43:1388-1395.
12. Gianaros PJ, Jennings JR, Olafsson GB et al. Greater intima-media thickness in the carotid bulb is associated with reduced baroreflex sensitivity. Am J Hypertens 2002; 15:486-491.

NÁZEV: *CHRONOBIOLOGY IN MEDICINE*
DEDICATED TO THE 85TH ANNIVERSARY OF PROFESSOR FRANZ HALBERG

EDITOR: *G. CORNÉLISSEN, T. KENNER, B. FIŠER, J. SIEGELOVÁ*

VYDAL
A VYTISKL: *NÁRODNÍ CENTRUM OŠETŘOVATELSTVÍ A NELÉKAŘSKÝCH ZDRAVOTNICKÝCH OBORŮ V BRNĚ*
VE SPOLUPRÁCI S MASARYKOVOU UNIVERZITOU BRNO

VYDÁNÍ: *PRVNÍ*

POČET STRAN: *146*

VYŠLO: *BRNO 2004*

VÝR. ČÍSLO: *97/04*

TIRÁŽNÍ ZNAK: *57-866-04*

ISBN 80-7013-412-7