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COSMIC INHERITANCE RULES: IMPLICATIONS FOR HEALTH CARE AND SCIENCE[1]

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ABSTRACT

Countering the trend in specialization, we advocate the transdisciplinary monitoring of blood pressure and heart rate for signatures of environmental cyclic and other variabilities in space as well as terrestrial weather on the one hand, and for surveillance of personal and societal health on the other hand. New rules (if confirmed novel laws) emerge as we recognize our inheritance from the cosmos of cycles that constitute and characterize life and align them with inheritance from parents. In so doing, we happen to follow the endeavors of Gregor Mendel, who recognized the segregation and independent assortment of what became known as genes. Circadians, rhythms with periods, τ, between 20 and 28 hours, and cycles with frequencies that are higher (ultradian) or lower (infradian) than circadian, are genetically anchored. An accumulating long list of very important but aeolian (nonstationary) infradian cycles, characterizing the incidence patterns of sudden cardiac death, suicide and terrorism, with drastically different τs, constitutes the nonphotic (corpuscular emission from the sun, heliogeomagnetics, ultraviolet flux, gravitation) Cornélissen-series .

LAWS AND RULES

Between 1856 and 1863, Gregor Mendel cultivated and tested some 29,000 pea plants. Between 2000 and 2008, Jarmila Siegelova's Brno team collected some 73,888 sets of systolic (S) and diastolic (D) blood pressure (BP) and heart rate (HR) measurements. With his 29,000 peas, Mendel collected evidence for the laws of heredity: segregation and independent assortment. Siegelova's some 73,888 cardiovascular sets, and these and her earlier proceedings, led to the rules of cosmic inheritance, which document the congruence (similarity

1 Dedicated to the senior author's daughter Francine Halberg on her 57th birthday, June 28, 2009.

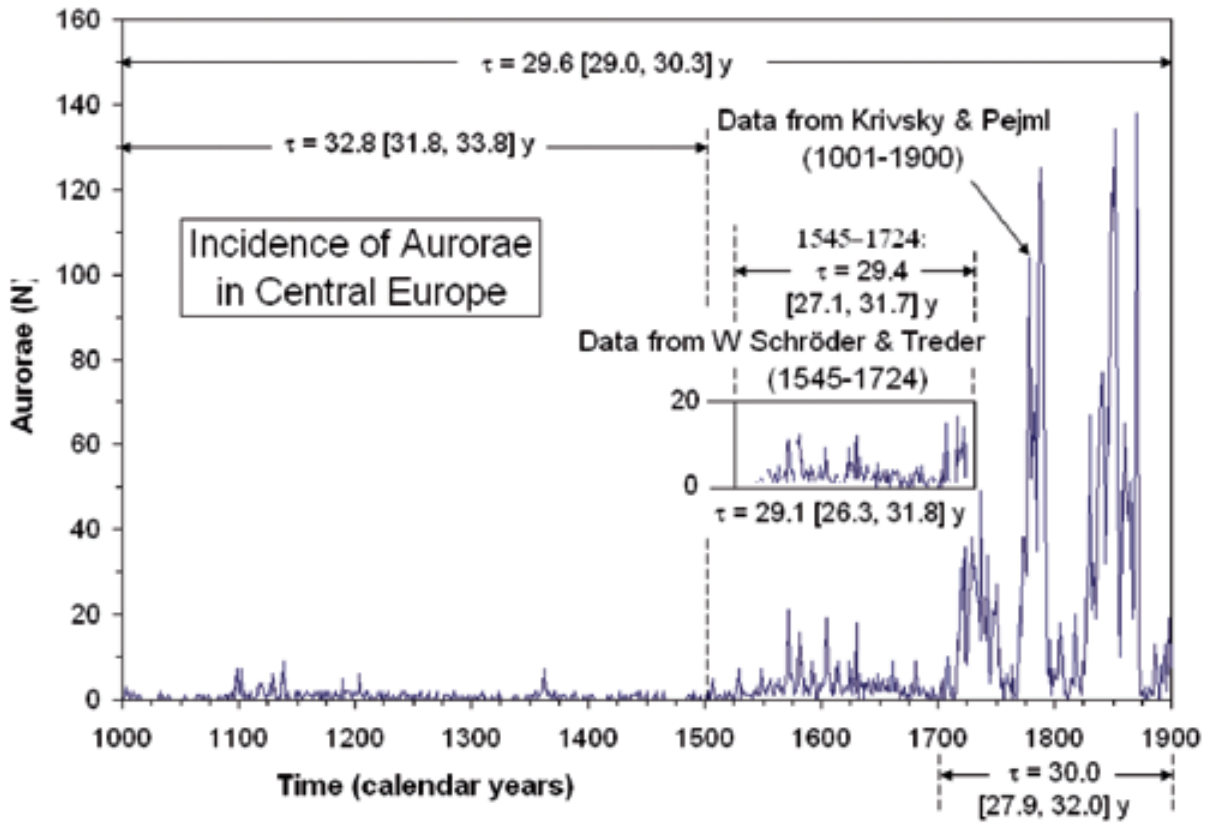

Figure 1
In a series of northern lights covering 900 years, a transtridecadal cycle is seen insofar as the CI, but not the point estimate of the period, τ, extends beyond 30 years. Data analyzed were questioned with the implication that their analyses may be invalid, even though they had yielded a result close to the anticipated finding. In view of that outcome and the observation that a statistically significant result in noisy data in our opinion constitutes a stronger evidence than the corresponding result in data with little or no noise, it seemed worthwhile to pursue the problem further. For part of the span analyzed, another set of data, regarded as more reliable [44], happened to be available. This afforded the opportunity for comparative analyses of that part of the 900-year span, 1545–1724, for which data deemed reliable were also available. It turned out that the extended cosinor yielded for the span of Schröder and Treder (1545–1724), in the latter's data, a component with a τ of 29.1 years and in the corresponding span from data of Krivsky and Pejml a τ of 29.4 years, as seen from this figure, which also shows that the CI of Schröder and Treder extending from 26.3 to 31.8 years overlies that of Krivsky and Pejml extending from 27.1 to 31.7 years. © Halberg.

characteristics such as the period or phase of a spectral component in two or more concomitantly sampled time series of variables with overlying or overlapping confidence intervals) and selective assortment of cycles around and in us. In monitoring the solar wind's signatures, we follow the aging Mendel: about (~) 13.5 months before his death Mendel considered an association of solar activity with worldwide auroras, searching for what is now recognized as the solar wind, i.e., particle radiation from the sun.

Mendel did this 124 years before we learned on a 16-year series of SBP from an elderly man (FH) that the same solar wind's far-transyear (1.2 years ≤ [τ - CI] < [τ + CI] < 1.9 years, τ {period}, CI {95% confidence interval}) and cis-half-year (of ~5 months length) are built into us as wobbly cycles amplified in the presence of a counterpart in the solar wind, dampened in the absence of this counterpart but not lost in us when the corresponding τ is no longer detected in the solar wind [2]. Thus, the Brnoese, Minnesotan, and other BIOCOS (project aimed at studying biological systems in their cosmos) monitoring is the aging meteorologist and epidemiologist Mendel's legacy. It has led to a complement to the two laws of genetics. When we align Mendel's inheritance from our

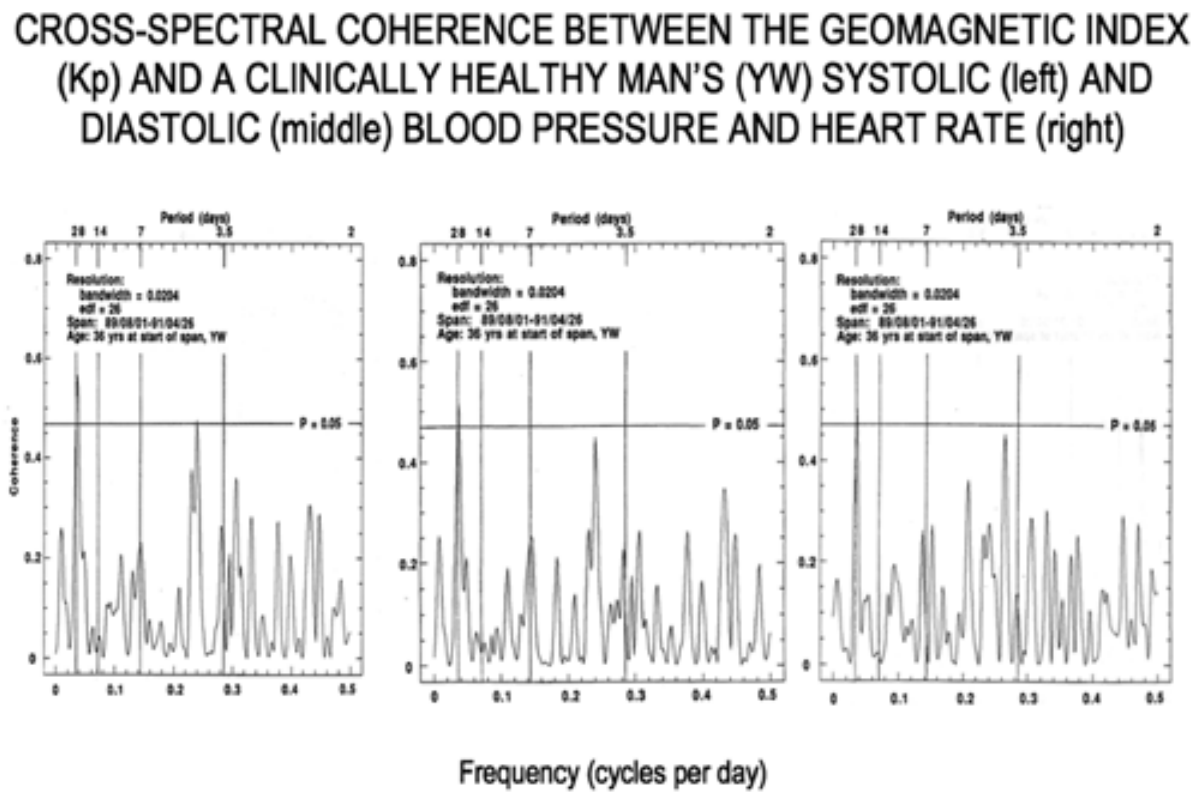

Figure 2
Global cross-spectral association of a man's circulation with the planetary geomagnetic disturbance index Kp. © Halberg.

immediate parents (i.e., the segregation and independent assortment of genes) with the manifold cycles one inherits from the sun and the broader cosmos, that is when we turn to cosmic inheritance, the two new rules are, first a wide transdisciplinary spectrum of congruent τs around and in us, with their congruence defined and documented by overlapping or overlying CIs of their τ, and second (if not quite independent assortment), then selective congruence, among different environmental and biospheric variables at various frequencies characterizing both.

Thus, we find that the HR of an adult (RBS) between 20 and 60 years of age is congruent with the ~33-year BEL cycle in Wolf's relative sunspot numbers (E. Bruckner, Ch. Egeson, W.J.S. Lockyer and R. Wolf were nineteenth-century astronomers), while his BP is congruent with an ~didecadal Hale cycle in the polarity change of the same Wolf numbers [3]. Two variables of the same circulation are locked into different environmental cycles. Selective congruence is again documented by overlapping or overlying CIs of the τs of heliomagnetics gauged by solar wind speed, geomagnetics gauged by the antipodal index aa and the estimation of 1 minute, a mental function, among others, in the transyear region of the spectrum of RBS. It is selective in that at some frequencies all three variables are congruent, or only two variables out of three at other frequencies, or at still other frequencies there is a statistically significant spectral peak in a biospheric or other variable with no congruent counterpart in other time series of the same variable, of other related variables (no intradisciplinary congruence) or in variables of other disciplines (no transdisciplinary congruence).

By the same token in the circaseptan spectral region, BP and HR are locked into different helio- and geomagnetic variables of an elderly man (GSK) [2]. An assortment of the congruence of different physiological variables with the same environmental cycle is also encountered in another elderly man (WRB) [4]. In the parasemiannual spectral region of RBS, different variables of his circulation can again show a different spectral behavior, as they do in a young man (DG) [5]. When congruence involves nonphotic invisible cycles in the environment and conditions such as human individuals' and

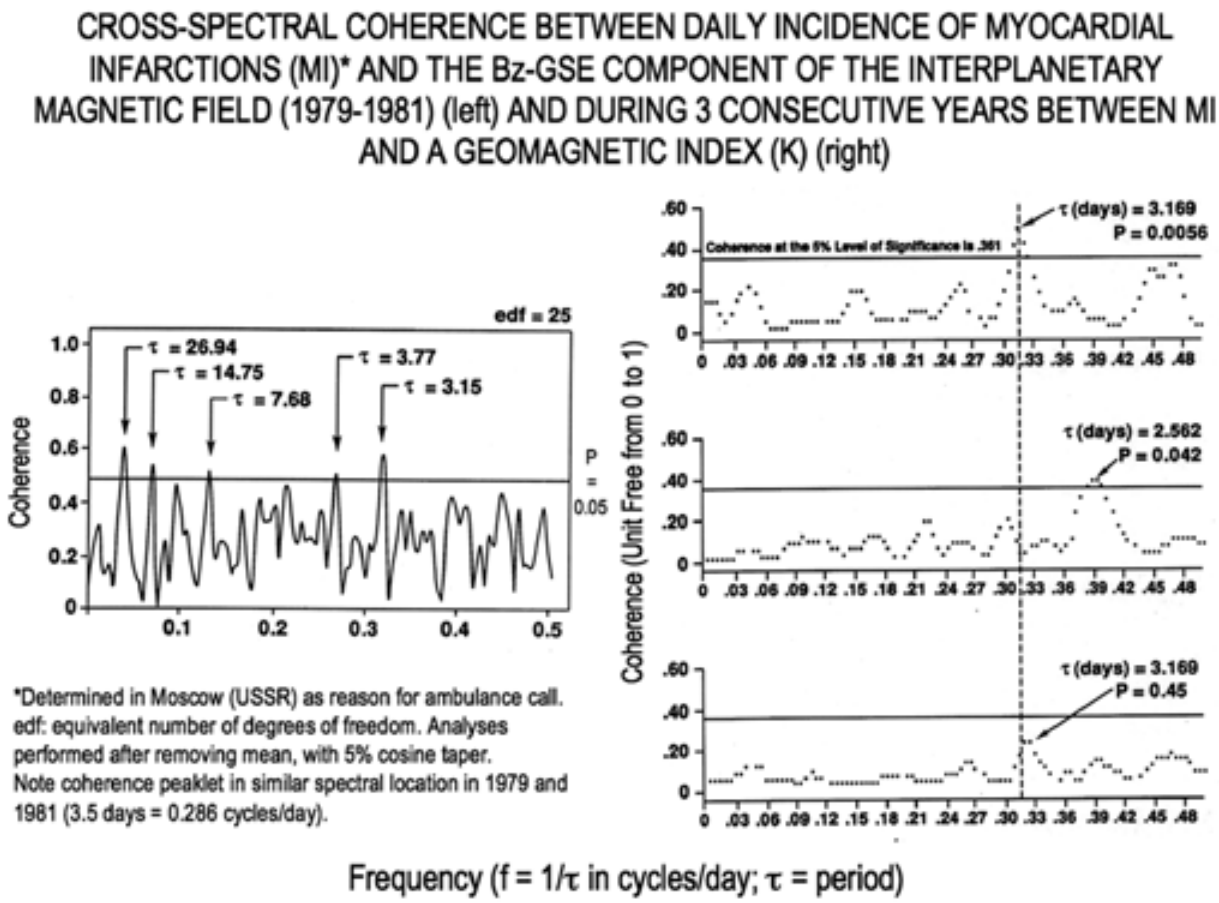

Figure 3
Global cross-spectral association of the incidence of myocardial infarctions in Moscow (1979–1981) and the Bz-GSK component of the interplanetary magnetic field. © Halberg.

populations' illnesses such as sudden cardiac death and terrorism, respectively [1, 6, 7], it gains in interest and justifies in itself the monitoring of the congruences of solar activity in the human circulation. But the justification also stems from what the given person can learn immediately for his/her health care.

In these proceedings, a physician-scientist (YW) [8] and his son, starting as a child and ending as an adolescent (FW) [9], set an example of family monitoring of BP and HR, the father providing for more than two decades, mostly half-hourly around-the-clock measurements, the son surveilled half-hourly for the first 40 days of his life [10] and self-measuring in childhood and during adolescence once a day for 8 years [9]. Thus, YW gathered evidence for a chronobiologically and longitudinally examined development (in well over two decades of his half-hourly around-the-clock measurements) of his high BP, MESOR-hypertension (MH), which is reversible, absent on Sundays and during a vacation spent at home [8]. The MESOR (M) is a Midline-Estimating Statistic Of Rhythm assessed parametrically by the fit of a 24-hour cosine curve. YW's M detects abnormality, MH, when the complementary non-parametric approach by stacking data does not (yet) find it in the prediction interval for single values, the "normal range" of peers of the same gender and age.

YW documents that MH can persist in his systolic (S) BP when it is cleared from the diastolic (D) BP. On the average, he shows no abnormality on Sundays and during a vacation. By intervention, with timed dietary measures or exercise, and by continuing monitoring he may provide more longitudinal data to reverse the workday elevation of the SBP MESOR as well, rather than to find an eventual excessive elevation of the SBP and/or DBP also on Sundays and on other days away

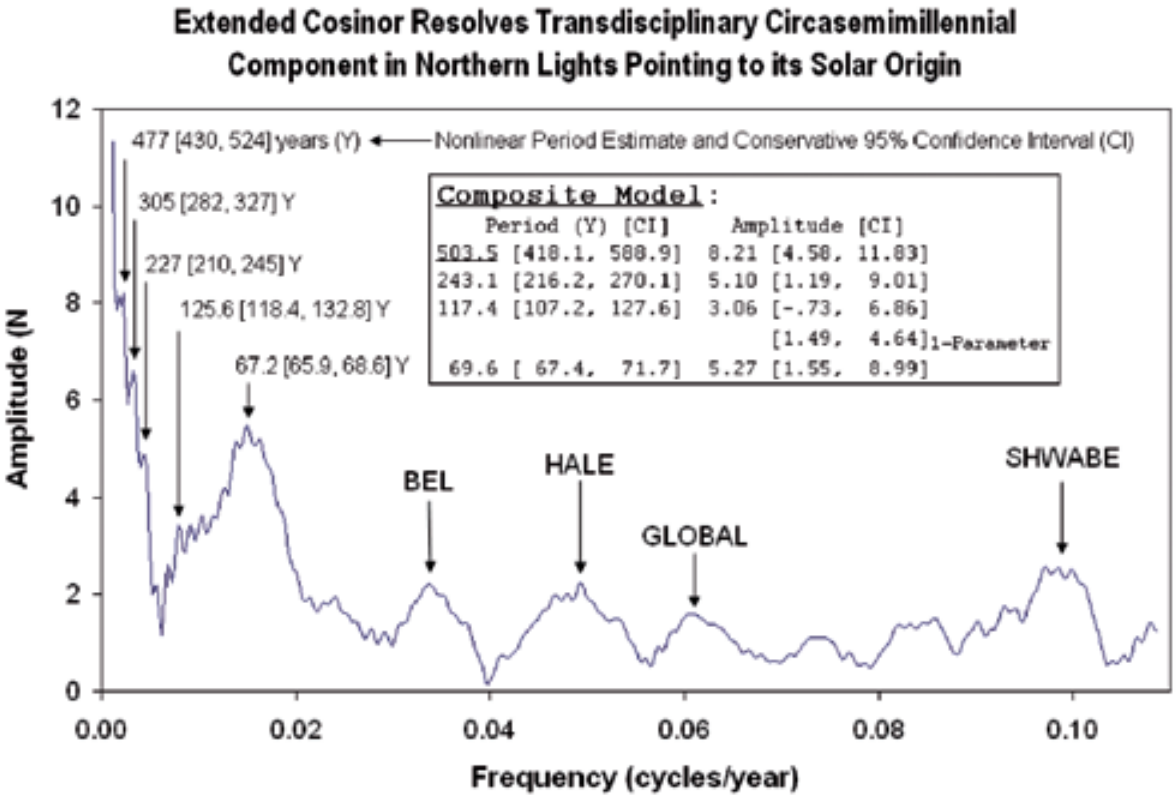

Figure 4

Data from J. Strestik (1001–1900). Results to be interpreted with caution since the average number of yearly observations increased as a function of time, likely in relation to technological observational progress. Data inspection finds about 3 different spans: the earliest with fewer observations, the middle one with more observations, and the more recent with considerably more observations. Trial periods: 500, 300, 200, 120, 70. Nonlinear results (each fitted separately): 477 [430, 524], 305 [282, 327], 227 [210, 245], 125.6 [118.4, 132.8], 67.2 [65.9, 68.6]. Composite model failed completely prompting removal of 300-year trial period. Results from concomitant fit with conservative limits are in years:

503.5 [418.1, 588.9] A=8.21 [4.58, 11.83]

243.1 [216.2, 270.1] A=5.10 [1.19, 9.01]

117.4 [107.2, 127.6] A=3.06 [-.73, 6.86] [1.49, 4.64]$_{1P}$

69.6 [67.4, 71.7] A=5.27 [1.55, 8.99].

Since the conservative 95% confidence limits of the 117.4-year period overlap zero, 1-parameter limits (1P) are also given in this case. Periods of 243.1 and 117.4 are possibly 2nd and 4th harmonics of 503.5, describing waveform. © Halberg.

from work. In the manipulation of sodium intake, it seems important to realize great inter-individual differences, and further the importance of circadian stage, that can both lead to decreases, rather than increases in BP in response to sodium. Alternatively, if progression cannot be stopped and the BP-M is not normalized by the absence of work, it will be of interest to see whether the parametric and the nonparametric approach reveal a concomitant DBP and SBP failure to reverse to normality on weekends or do so in a sequence, and if the latter is the case, which of the BP remains abnormal on Sundays first. Clearly, the over two decades of monitoring by YW do not suffice to draw full inferences concerning the development of a human MH on weekends as well as on workdays. In these proceedings, YW's contribution to the development of workday MH is accompanied by the contribution of three elderly men (WPB, GSK and FH) who are all treated for MH and whose data on the one hand indicate the need for continued surveillance to avoid circadian hyper-amplitude tension

(CHAT, 11). FH's follow-up record shows the need for continued surveillance to detect and, if so, to attempt to eliminate CHAT and further to validate the extent of success – e.g., by the use of coenzyme Q10 (Q-Gel) with the evening meal [12] in eliminating 7-day CHAT – and to recognize any failure, e.g., to eliminate 24-hour CHAT (data not shown).

The approach is practiced as yet by a minuscule minority in noninvasive automatic cardiology in the spirit of Theodore C. Janeway [13] and Frederic C. Bartter [14], who had to rely on self-measurement. Let us call this school chronomic, when it now aligns make-ups in time – chronomes – of an organism with those of the sun's variability, the original time structures or chronomes. Not only ~daily, ~half-weekly and ~weekly but many much longer τs have been found to characterize human physiology and pathology at the levels of individuals and societies, including ~5-month as well as ~6-month cycles, the former helio-, the latter geomagnetic in origin, with signatures in BP, HR, cardiac arrhythmia and sudden cardiac death, facts that must not allow their neglect. Yet practicable restrictions of focus at this time, primarily on ~24-hour rhythmic behavior during a week and on the relation of these circadian characteristics to half-weekly ones could provide novel information bringing millions of people into the loop of self-help for preventive care. The chronomic approach can be implemented by self-measurements and more readily and more densely, by self-help with automatic recording thanks to modern technology. It has accumulated, with gaps, half-hourly data for up to well over two decades yet as a minimum for seven days on sets of hundreds of individuals.

The data document day to day and other presumably normal variability in human BP and HR around the clock and lead to the construction of reference ranges and, in their light, to the finding of Vascular Variability Disorders (VVDs). These VVDs could be detected in current practice, as could be the fact that a good drug combination for BP-lowering, such as 12.5 mg of hydrochlorothiazide and 50 mg of losartan (Hyzaar), can exacerbate an SBP VVD (a circadian overswing, CHAT) and induce this DBP abnormality with one timing of its administration and eliminate CHAT with another timing, each tested for a month in the same patient [15]. The inferences drawn on the basis of long records comparing the same drug in the same dose, taken by the same person with a different timing in relation to awakening, revealing drastically different effects, constitute evidence that, sooner rather than later, could be considered by those who currently rely on their platinum standard of a single day's profile. A 24-hour record simply does not suffice for a prognosis when in the same young neurosurgeon, during his office hours (09:00–17:00), 77% acceptable measurements on one day alternate with 100% unacceptable (too high) ones on another day (16; cf. 17–21).

If in turn current health care remains homeostatic, still assuming, rightly or (we believe) wrongly that there is indeed a "true" BP and that this can be assessed, if not by single measurements than by single 24-hour profiles, some recent suggestions sound reasonable. In 2009, some advocate "... lowering BP in everyone over a certain age, rather than measuring it in everyone and treating it in some" [22]. A polypill containing three BP-lowering drugs, each at half the standard dose – a statin, folic acid, and aspirin – would serve the purpose of primary and secondary prevention [23]. Others [24] also "support the use of a 'polypill' to lower the risk of cardiovascular disease in people likely to be at risk (such as all people over the age of 55) without first checking their BP." This approach is logical and justified from the viewpoint of a health care based on pseudo-evidence that is based upon a few spotchecks including at best a single chronobiologically uninterpreted 24-hour profile, which checks a small fraction of a half-weekly or weekly cycle, as if the pulse were taken for just a small fraction of one cycle, i.e, a fraction of a second. When all VVDs requiring long-term surveillance are ignored, as is, for instance, the risk of a stroke in 6 years which can be elevated by an undiagnosed VVD from ~5% to 100% in one study [21], a polypill is an attractive and economical answer. But as we monitor with self-help, cost-free to start with at half-hourly intervals for 7 days and continuously monitor when abnormality is found, the merits of self-surveillance become obvious, also for checking on the demerits of the polypill or any other treatment, such as the induction and exacerbation of CHAT, on a substantial number of patients [15, 21].

The arguments that such monitoring is costly, complex and hence futuristic can be met with modern computer-aided technology by the demonstration that self-surveillance is possible and is now ongoing on a limited scale, and the complexity can be eliminated by the computer charged with all complex calculations and providing free and clear interpretations.

FOLLOWING MENDEL'S INTEREST IN THE AURORA

Human population and broader biospheric monitoring has a long way to go in the biosphere to match the information gathered on the aurora, where the record covers nearly a millennium, as analyzed in Figure 1 [25], which reveals a transtridecadal cycle also found in environmental temperature [26] in the sediments of an African lake [27] and in many other variables [3, 28–34].

According to Battersby [35], Galileo described the auroras as sunlight reflected in vapors rising from the earth. Battersby also writes: "In the late 1600s, Edmond Halley [1656–1742] was the first to correctly link the aurora to the Earth's magnetic field." According to Egeland [36], a close connection between the aurora and the geomagnetic field was found around 1730

by Hierter and Celsius. It is the more interesting to read in De Mairan's book [37] on that topic that he ruled out magnetism as underlying the aurora because "magnetic matter is constant". Had he pursued his earlier botanical observation [38] that the sensitive plant continued its sleep movements in continuous darkness, and had he measured geomagnetism concurrently, he could have found an ~24-hour cycle in both and would perhaps not have ruled out constant magnetism in the genesis of the aurora. It is the more interesting that in 1882 Mendel associated solar activity with the aurora. A transtridecadal cycle in this phenomenon was first reported for the past 500 years without specific comment by Sam Silverman [39].

In the case of yearly data on northern lights from a catalogue by Krivsky and Pejml [40], between the years 1001 and 1500 our meta-analysis (illustrated in Figure 1) shows a BEL. The linear-nonlinear extended cosinor (41–43) reveals a τ of 32.8 years, with the CI of this τ extending from 31.8 to 33.8 years and an amplitude with a CI that does not overlap zero (Figure 1, row 3). Also by linear-nonlinear extended cosinor, during the entire span from 1001 to 1900, a transtridecadal BEL τ is ~29.6 years long with a CI extending from 29.0 to 30.3 years and an amplitude with a CI only slightly overlapping zero, Figure 3C (bottom row).

While the quality of the data analyzed [40] has been questioned, there is the fact that the greater the noise in a time series, the more convincing is the validation of an anticipated result. This is indeed the case in Figure 1, which also shows our analysis of data deemed more reliable by Schröder and Treder [44]. When the analysis of the data of Krivsky and Pejml is restricted to the span of the data published by Schroeder and Treder, the results are in excellent agreement in Figure 1. For the interval 1500 to 1948, as noted, a scholarly analysis and review by Silverman [39] supports the hypothesis that a nonrandom albeit greatly variable transtridecadal pattern in solar activity includes a spectral component of 33.3 years in the power spectrum of monthly auroral occurrence, in keeping with the prior report of a climatic ~33-year cycle by Eduard Brückner [45], who fully realized the transdisciplinary importance of his wet/cold-dry/hot cycle and offered public lectures on that topic.

"[In any event, the aurora can be] more than a pretty display. These storms are caused by violent outbursts from the sun and can play havoc with satellites, scramble GPS signals, endanger astronauts and even blow power lines on Earth" [35]. This is as far as scientific journalism ventures, not yet to human physiology. The effect of storms in the biosphere is rarely considered by the public, but is of particular interest to neuroendocrinologists. It will be up to scholars of melatonin (46, 47), to further map the infradian challenges found in the record-holding test pilot, Dr. Robert B. Sothern (RBS), author of a comprehensive scholarly book in the field of rhythms [48]. RBS yielded a model for motivation and consistency and demonstrated, in a record of ~5 HR measurements around the clock now covering over 40 years, a transtridecadal cycle also found in Zürich (Wolf) sunspot numbers during the span covered by his data.

The BEL (3, 28–34) is one of the windows for nonphotic mostly invisible effects of the cosmos upon the biosphere. It also characterizes the aurora as the "greatest show on earth" for most of the past millennium, with associations in the biosphere. NASA's flotilla of satellites, named THEMIS, sent into space to analyze magnetic substorms as they happen and to understand the sequence of events that bring about the northern lights, promises to tell us more about the physical mechanisms involved, but should be checked for biospheric associations. Battersby writes: "During a geomagnetic storm there are typically several substorms, but how the two are connected is unclear. So far during the mission, solar activity has been low, but it should increase over the coming year or so, giving THEMIS a chance to watch a much larger storm unfold" [35]. We should concomitantly watch what happens in the biosphere as well. Long-term monitoring, such as that ongoing for years or decades is needed to learn about infradians in a number of frequency windows that also include a transyear [2, 49–52] and cis-half-years [2, 53], the latter involving steroids and melatonin [54] in mediating effects of our cosmos, perhaps via the eyes [55].

Cycles with a τ of over 3 decades' length now documented in an individual's, RBS', physiology and psychology are reminiscent of the documentation by W.J.S. Lockyer [33] that these rather long cycles may stem from changes in the length of the sunspot cycle generated by the cosmos and reflected in the interplanetary magnetic field. We can look with hindsight at what could have been done in dealing, for instance, with sunspots by individuals who could not observe too many ~35-year cycles. They, like ourselves, could probably measure not much more than part of a single adult or part of an elderly 35-year cycle. If Carl Friedrich Gauss' least-squares had been available in Horrebow's time, i.e., in the 18th century, he did have enough data to objectively document, with the cosinor method by least-squares, a cycle of ~9 years in his observation span (54).

In either case, for the variable sunspot cycle at the times in history when it happened to be studied in the 18th and 19th centuries, the necessary length of the time span needed for rejecting the zero-amplitude assumption and to get reliable estimates of parameters was not longer than a single cycle's length [54]. Without extrapolating beyond the scope of the specific data analyzed, we find the same success for the transtridecadal BEL [3, 28–34]. These precedents should encourage neuroendocrinologists, among many others, to sample

systematically for very long spans, since some if not many of the novel infradian rhythms, such as cis-half-years and trans-years, may well be modulated by transtridecadal cycles. The latter have implications as an aspect of the climate for global temperature, that as Brückner lectured have contributed to population dynamics and many other human affairs [30, 45].

DISCUSSION

The challenge of circadians is complemented by the greater challenge of infradians that require lifetime monitoring, preferably by unobtrusive and generally affordable methods yet to be developed in nanotechnology. Before this goal is achieved, data collection by available automatic instrumentation, as used by FH, YW, DG and FW, or manually by RBS, FW and WRB, has to be relied upon so that infradian focus can be directed at major problems of society such as those documented elsewhere [6]. Data for this critical monitoring of solar activity could be contributed by humans, rendering their health care cost-effective for stroke and other severe vascular disease prevention by self-monitoring and obtaining data analyses automatically and cost-free from a website, as BIOCOS had done since 1991 (56; cf. 57), analyzing physiological data, Figure 2, as well as those accumulating in archives, Figure 3. This website could thus serve for service and research, both medical and transdisciplinary, and is being constructed by the Phoenix Study Group, composed of volunteering members of the Twin Cities chapter of the Institute of Electrical and Electronics Engineers (http://www.phoenix.tc-ieee.org). In the interim, analyses are available from the international project on The Biosphere and the Cosmos, BIOCOS (corne001@umn.edu).

CONCLUSION

All known τs in solar activity up to ~500 years length that may seem to be negligible peaklets in the aurora, Figure 4, characterize human individuals' and populations' variables including, as far as an individual's lifespan allows, HR, BP, body weight and mental performance in health, and have signatures in societal variables including morbidity and mortality, as had long been suggested, mostly without any inferential statistical documentation of the uncertainties involved. These uncertainties (CIs) can now be mapped with their well-known τs, such as the Horrebow-Schwabe decadal cycle, the Makarov and Sivaraman global solar cycle of ~17 years, Hale's ~didecadal bipolarity cycle, and for what we rediscovered as the Brückner-Egeson-Lockyer (BEL) cycle which is reflected in the total solar flare index, in solar wind parameters of the interplanetary magnetic field and in many human physiological and societal variables including 2,556 years of international battles, thousands of years of tree rings, and cave temperatures, and in the emergence on different continents of outstanding physicians, poets and historians (the emergence of outstanding individuals a contribution by Prof. Miroslav Mikulecky) (58; cf. 59). A major transdisciplinary point is that the presence of a biospheric counterpart may support if not validate the corresponding environmental counterpart and vice versa. The eons of evolution have likely contributed to the biospheric finding, whereas, e.g., any peaklets found in Figure 4 by the cosinor analysis of a 900-year series of the aurora poses legitimate doubts about possible artifacts. Such doubts are always part of science and the limitations of the kind of data available are ever present; but when a peaklet found in the aurora at a trial τ of ~500 years has biospheric counterparts, as indeed it does, it gains greatly in transdisciplinary interest.

The immediate now inferentially statistically documented health effect of new ~5- or ~16-months cycles relate to sudden cardiac death, suicide, crime and terrorism [6], in which nonphotic cycles characterizing the solar wind can altogether replace photic cycles such as the yearly (circannual) change. While we develop unobtrusive, affordable monitoring tools for stroke and other hard disease prevention, and these tools are already available as prototypes for general use, let us also analyze the same data of our physiology and health departments' archives about our pathology for monitoring space weather. While we contribute greenhouse gases, it is necessary to know climatic changes as a result not only of human activity but of the external influences as well. The most powerful such influence is solar variability, predictable only insofar as it is cyclic. What is new is that we can gauge that variability by that of BP and HR. Let us not fly blind with respect to both solar and human variability as these affect individual and societal health. A polypill will neither explore the risk of societal disease nor will it lower this risk. If, perhaps, it has merit in clinical trials, like any other antihypertensive treatment, it will represent increased risks that require self-surveillance, which is the essential recommendation of the accompanying set of 19 presentations.

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BLOOD PRESSURE, HEART RATE AND MELATONIN CYCLES SYNCHRONIZATION WITH THE SEASON, EARTH MAGNETISM AND SOLAR FLARES

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ABSTRACT

Three spectral components with periods of about (~) 0.41, ~0.5 and ~1.0 year had been found with serially independent sampling in human circulating melatonin. The time series consisted of around-the-clock samples collected for 24 hours at 4-hour intervals from different patients over several years. Some of these components had been found to be circadian stage-dependent, the daytime measurements following mostly a circannual variation, whereas a half-year characterized the nighttime samples. The latter were incorporated into a circasemiannual map. The relative brevity of the series prevented a check for the coexistence of all three spectral components, even if each component seemed to have a *raison d'être*. In time series of transdisciplinary data, a 1.00-year synchronized component is interpreted as representing the seasons. The half-year may qualify the circannual waveform, but it is also a signature of geomagnetics. An ~0.41-year (~5-month) component is the signature of solar flares. It has been called a cis-half-year (cis = on this side of a half-year) and may be detected only intermittently. Charles L. Wolff predicted the existence, among others, of ~0.42- and ~0.56-year components as beat periods of rotations at different solar latitudes.

The multiple components characterizing circulating melatonin could also be found in a (to our knowledge unique) data set of a clinically healthy scientist (RBS). Herein, we focus on vascular data self-measured by RBS as he aged from ~20 to ~60 years. A multi-component model consisting of cosine curves with periods of 0.41, 0.50 and 1.00 year was fitted to weekly means of systolic (S) and diastolic (D) blood pressure (BP) and heart rate (HR) collected ~5 times a day over 39 years by RBS. All three components can coexist for a while, although all of them are nonstationary in their characteristics and come and go by the criterion of statistical significance.

Intermittently, BP and HR are synchronized selectively with one or the other aspect of RBS' physical environment, namely the seasons (at ~1.0 year), earth magnetism (at ~0.5 year) and/or solar flares (at ~0.42 year). Cosmic-biotic transfer of information, albeit hardly of energy (the biospheric amplitudes are very small) may be mediated in this set of frequency windows. As found earlier, RBS' circulation is also frequency-trapped environmentally in multidecadal windows, HR being locked into the transtridecadal Brückner, or rather Brückner-Egeson-Lockyer, BEL sunspot and terrestrial weather cycle, while his BP follows Hale's didecadal cycle in the changing polarity of sunspots.

The ~0.41-year HR cycle may be associated with changes in solar flares, the cis-half-year amplitude of HR showing a cross-correlation coefficient of 0.79 with the total solar flare index (from both solar hemispheres) at a lag of ~3.2 years. The superposed time courses of these two variables indicate the presence of a shared Horrebow-Arago-Schwabe sunspot cycle of ~11 years, the cis-half-year in HR being more prominent after the total solar flare index reaches its ~11-year peak. Differences in the time-varying behavior of BP vs. HR are also described.

AN ANALOGY BETWEEN EXTERNAL SCRUBBING AND SELF-SURVEILLANCE OF INTERNAL RHYTHMS

With respect to preventive measures for maintaining cardiac and mental health and preventing morbid events such as strokes and criminality in individuals and terrorism in society, we are in the same situation today as Ignaz Semmelweis [1] and Oliver Holmes [2] were with respect to antisepsis. In our case, a broad spectrum of rhythms in us and around us, albeit already mapped, has not yet been recognized in terms of pertinence to everyday life. Like circadians [3–5], now a fashion in molecular biology, the many different more or less periodic extra-circadians [6] evolved and were adaptively built into human physiology under the influence of the nonphotic cycles of our cosmos. Infradians are apparent in military-political affairs, including aggression, notably crime, international battles and terrorism, in economics, in opinion polls, in education and, most important, in health and disease, including infection (7–10). Like circadians, infradians also tip the scale between life and death.

DOUBLE PURPOSE OF PHYSIOLOGICAL SURVEILLANCE: SELF-HELP IN HEALTHCARE AND PHOTIC-NONPHOTIC ENVIRONMENTAL MONITORING

Chronobiologically interpreted systolic (S) and diastolic (D) blood pressure (BP) and heart rate (HR) monitoring detects pre-hypertension, pre-diabetes, and a pre-metabolic syndrome in Vascular Variability Disorders (VVDs), as conditions of increased vascular disease risk [11–14] and as complications of a (reliably diagnosed) MESOR-hypertension, a VVD in its own right. Another VVD is an excessive circadian amplitude of BP (CHAT, short for Circadian Hyper-Amplitude-Tension). This condition carries a risk of cerebral ischemic event greater than a high BP. It can coexist with other VVDs to form Vascular Variability Syndromes (VVSs). VVDs are not being screened for in our current conventional health care system. Once diagnosed, some VVDs can be treated.

It is not yet generally known that in the human newborn, variables such as BP and HR have infradians such as an about-weekly component, which is usually more prominent than the 24-hour variation, awaiting testing as a gauge of risk. It is also not generally known that the incidence patterns of sudden cardiac death (ICD10, code I46.1) is characterized by ~5-month (cis-half-year) and ~17-month (far-trans-year) components, cyclic signatures of space weather, in the absence of yearly changes, notwithstanding the large difference between Minnesota's mid-continental winters and summers. A host of spectral components is shared between natural physical environmental variables, such as the solar wind and other aspects of space weather, and biological variables, such as those of the human circulation, electrical accidents of the heart [15], and suicides [16]. They are said to be congruent when the 95% confidence intervals (CIs) of their (shared) periods are overlying or overlapping. (Congruence is a similarity in characteristics such as the period or phase of a spectral component in two or more concomitantly sampled time series of variables when 95% CIs are overlying or overlapping.)

When cardiovascular and mental health and their relation to the cosmos are viewed in 40 years of self-measurements by RBS, there is selective congruence of the ~5-month component between RBS' HR and the total solar flare index (SFI). An ~0.41-year cis-half-year component in HR is modulated by solar flares, and both solar flares and the cis-half-year amplitudes of RBS' HR show the Horrebow-Arago-Schwabe pattern of sunspot numbers (Figure 1, left). Their cross-correlation function (Figure 1, section D) reaches a maximum of r=0.79 at a 3.16-year lag, raising the question whether the effect of solar flares is direct or indirect, perhaps mediated by terrestrial magnetism. Congruence of an ~33-year cycle between RBS' HR and Wolf's sunspot numbers (Figure 2) was reported earlier [17], as were other congruences of RBS' HR and BP with space weather (Figure 3).

HORREBOW-ARAGO-SCHWABE MODULATION OF CIS-HALF-YEAR IN AND AROUND US

Both solar flares (Figure 1, left, top and bottom) and Wolf sunspot numbers (Figure 1, left, middle) are characterized by a prominent ~11-year cycle. The prominence of the cis-half-

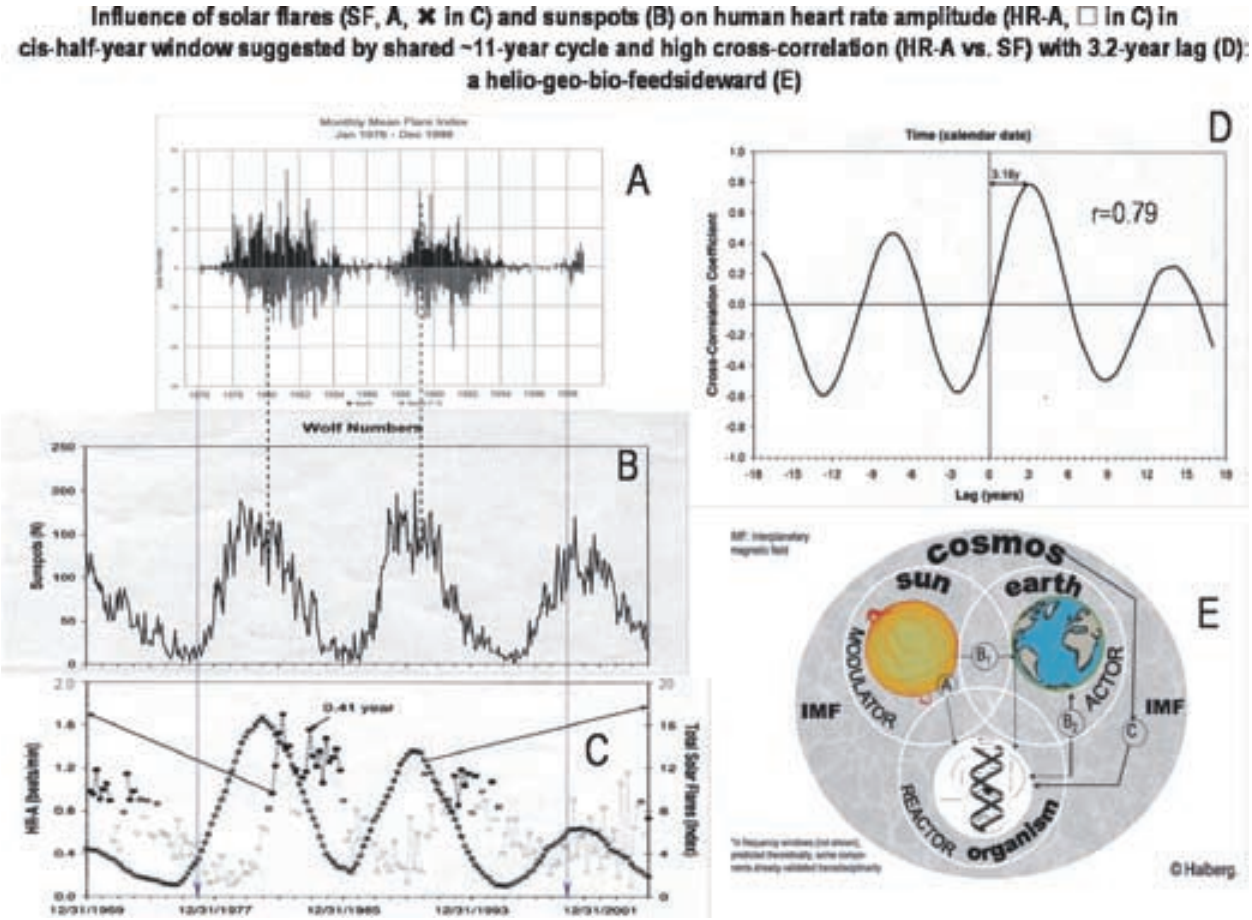


Figure 1

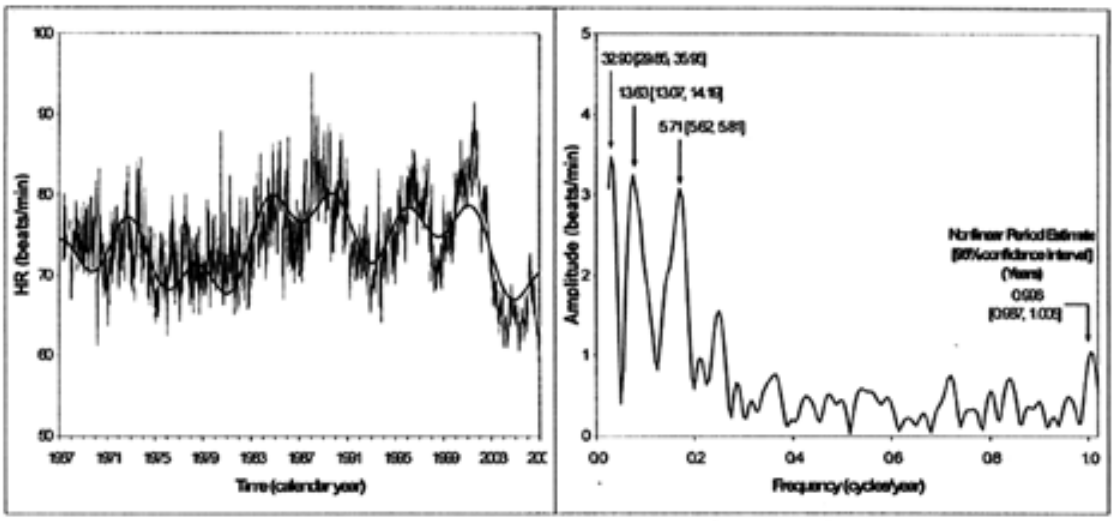
Horrebow-Arago-Schwabe pattern of solar flares (top and bottom, left) and of Wolf numbers (middle, left) is shared with changes in cis-half-year amplitude of RBS' HR (bottom, left). The cross-correlation function of the latter with the total solar flare index reaches a maximum of 0.79 at a 3.16-year lag (top, right). © *Halberg*.

year in RBS' HR, gauged by the 0.41-year amplitude estimated over a 4-year interval displaced in 2.5-month increments, also follows a similar ~11-year cycle (Figure 1, left, bottom). It is detected with statistical significance (filled rectangles) only part of the time, usually following a peak in solar flares and sunspot numbers. The cross-correlation function between the cis-half-year amplitude of HR versus the total solar flare index reaches a maximum of 0.79 at a 3.16-year lag (Figure 1, right, top). Whether the effect is direct or indirect should be examined further, beyond the congruence of periods and phases, as they change with time, against the background of already demonstrated prior global congruences at a trial period of ~33 years, Figures 2 and 3. Congruences can be selective, as noted in Figure 3: RBS' HR displays a transtridecadal congruence with Wolf numbers, while RBS' BP responds to Hale's sunspot bipolarity cycle.

CAN BIOSPHERIC BEHAVIOR TEACH PHYSICAL LESSONS?

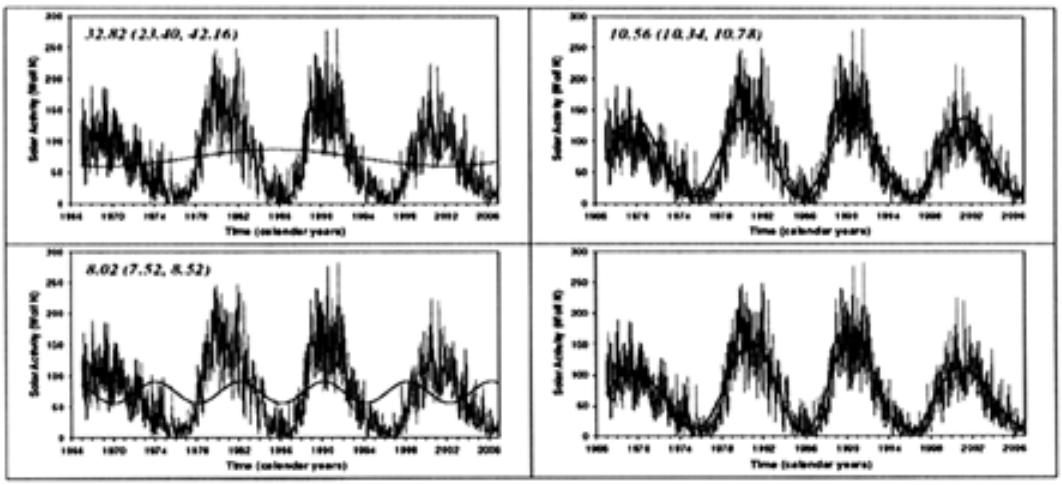
The original task was to examine the degree of ubiquity and, in this sense, the consistency in different variables, if any, of an ~0.42-year cis-half-year component. It had been found to characterize sudden cardiac death (ICD10, code I46.1) in Minnesota and in a number of other geographic locations (15; cf. 18–20), Figure 4. It was also detected in the SBP of an elderly subject (FH) during nearly 16 years of monitoring, at 70–86 years of age [15], and in a 10-year DBP record (analyzed by gliding spectrum) of another elderly man (GSK) [21]. The 39-year BP and HR record of RBS, at 20–59 years of age, lent itself well to the examination of the cis-half-year as it may change as a function of time.

BEL Cycle in Human Heart Rate during 40 Years *



*Weekly averages of data from RBS, clinically healthy man, 21 years of age at start of self-measurements 5-7 times per day during 1967-2006 (N=1978). Transtridecadal Brückner-Egeson-Lockyer cycle (BEL) of 32.9 years in human heart rate of RBS given with its 95% confidence interval in the spectral window (right), derived from original data shown on the left with the fitted model. Note that numerically the BEL cycle has the largest amplitude in the window examined, while it is the smallest in Figure 4 in Zürich numbers. BEL cycle is dominant while Zürich sunspot numbers below show only a week statistically significant counterpart. © Halberg.

Wolf Numbers During a 40-Year Span of Physiological Monitoring Show an About 33-Year Cycle (top left), Also Seen in Heart Rate Data (Latter Not Shown)*



Composite 3-component model fitted to data (bottom right), with contribution by each separate component also shown (top and bottom left) with nonlinearly resolved period (in years) and 95% confidence interval in person. Minor transtridecadal BEL aspect of Zürich sunspot numbers, top left, as compared to other components with much larger amplitude (top right and bottom left) and by contrast to the prominent BEL in heart rate in *RBS (clinically healthy man, about 20.5-years of age at start of monitoring) shown in Figure 3. Entire model shown at the bottom on the right. © Halberg.

Figure 2

Congruences were reported earlier [17] at a period of ~33 years between RBS' HR and Wolf's sunspot numbers. Whereas this component is rather weak in the physical variable, it is much more prominent in HR, as apparent from the least squares spectra. © *Halberg*.

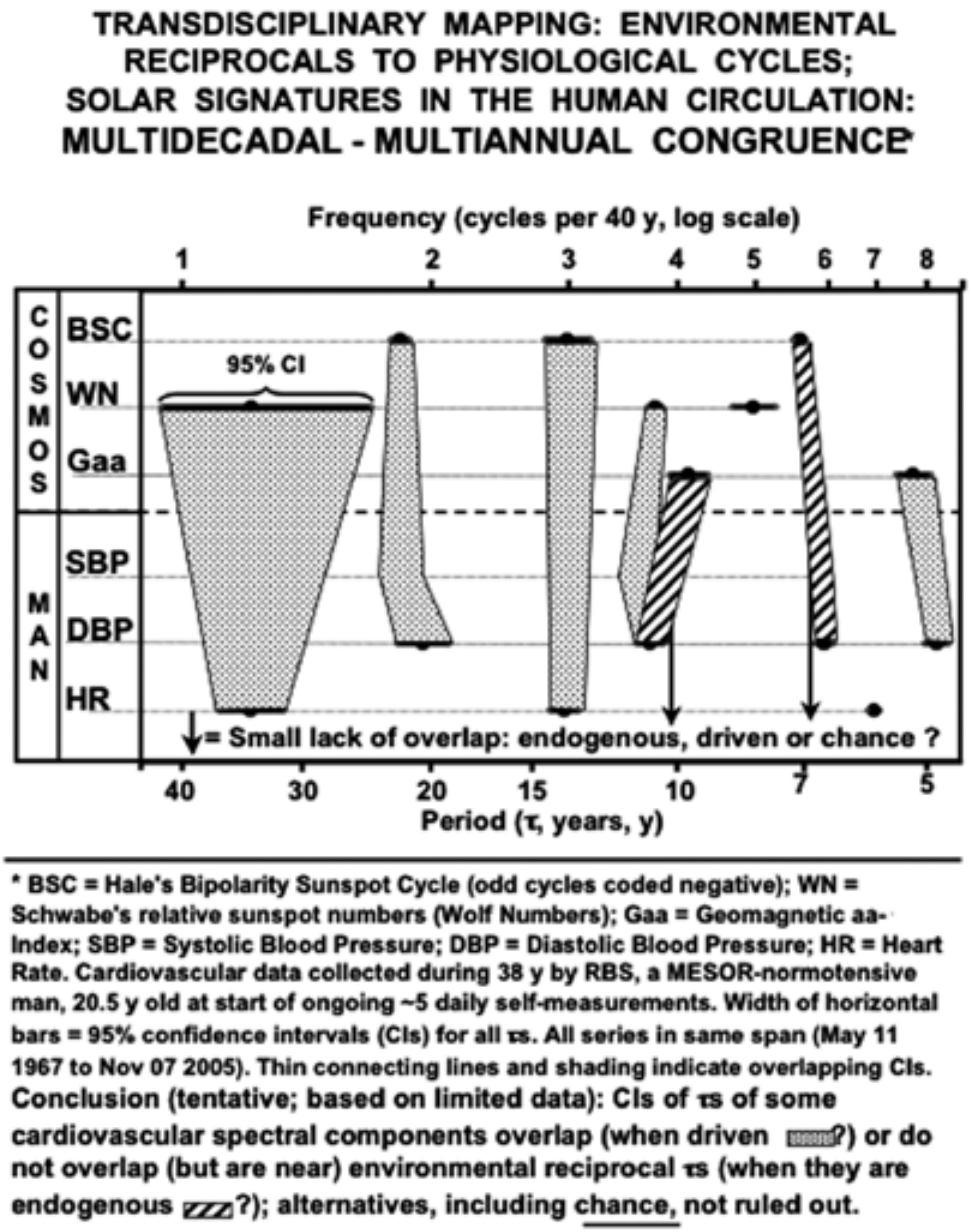


Figure 3
Summary of relative congruences between RBS' HR and BP and nonphotic environmental variables, including the one shown in Figure 2 (top left). Note a didecadal congruence between RBS' BP and Hale's bipolarity cycle of sunspots. © *Halberg*.

LOOKING FOR INTERMITTENTLY COEXISTING CYCLES

Linear least-squares spectra of weekly means of SBP, DBP and HR of RBS showed only a small peak at a period of ~0.42 year that was not statistically significant, contrasting with its detection in the BP of two elderly men (FH and GSK). The task was to determine whether the cis-half-year was definitively absent in RBS during his 39 years of self-measurements or whether its detection was obscured by the presence of other larger neighboring spectral peaks and/or by its nonstationary Aeolian nature. (The intermittent and otherwise nonstationary waxing and waning in amplitude was dubbed

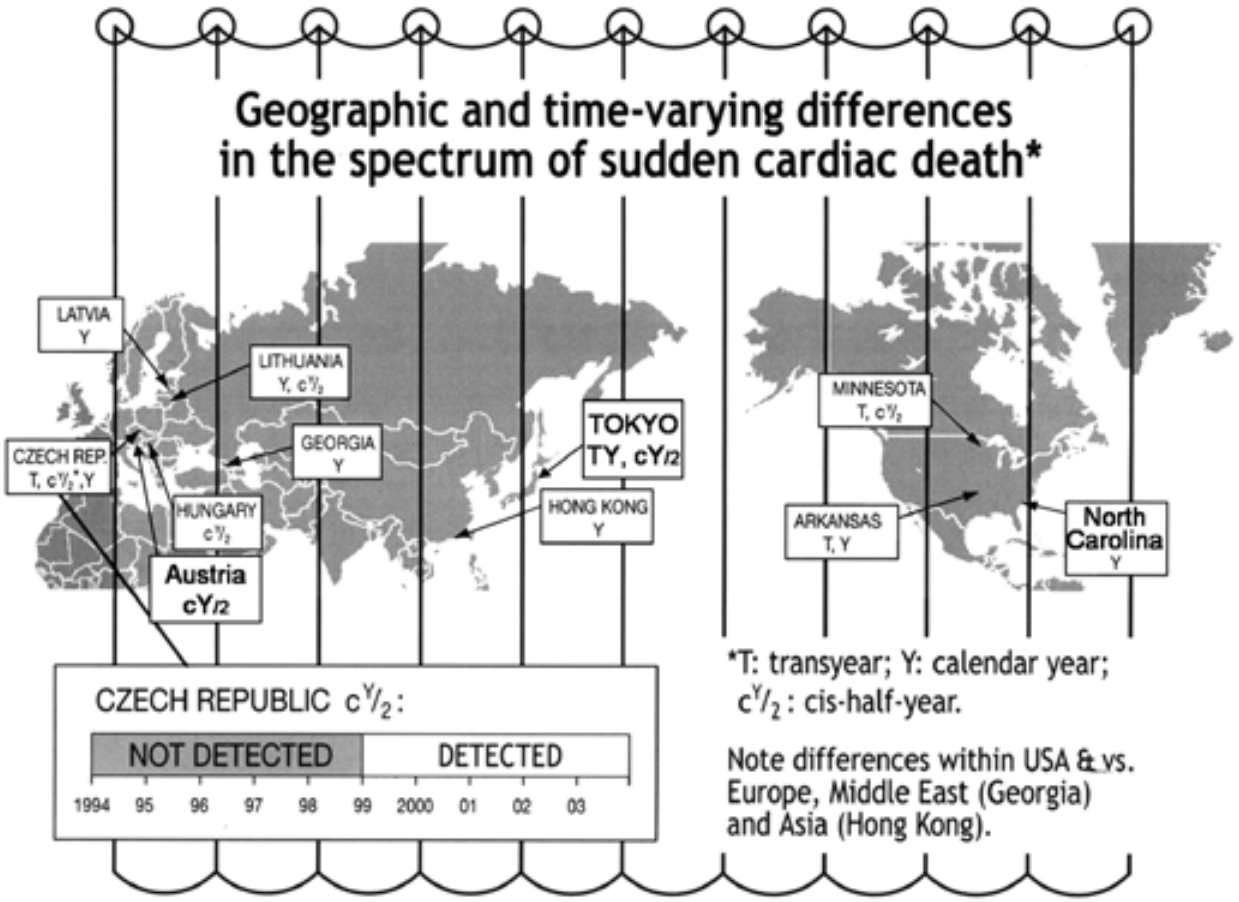


Figure 4
A cis-half-year (cY/2) had been found in the incidence patterns of sudden cardiac death in Minnesota [15] and in some other geographic locations, as summarized in this map. © *Halberg*.

"Aeolian".). Prominent yearly and half-yearly variations indeed characterized the BP and HR of RBS. The half-year may be an expression of the non-sinusoidality of the circannual component, but it may also represent the expression of a geomagnetic signature of its own right. The ~0.42-year cis-half-year was anticipated from its detection in two other long-term series, being congruent with solar flares, where this component was documented by Rieger et al. [22] and others, Table 1. An ~0.56-year component in solar activity, also of interest as another beat period of the solar rotation according to Charles L. Wolff (23, cf. 16), remains beyond the scope of this analysis. In order to answer the above question, multiple-component serial sections were carried out, including with the 0.42-year cis-half-year the other two major neighboring components with periods of 0.5 and 1.0 year. Specifically, a 3-component model was fitted by cosinor, consisting of cosine curves with periods of 1.0, 0.5, and 0.41 year (or 8766, 4383, and 3615 hours), to data in a 209-week (~4-year) interval displaced throughout the time series by increments of 11 weeks (~0.21 year or a half cis-half-year cycle).

Like many other nonphotic components, the cis-half-year in the BP and HR of RBS was found to be present only intermittently and with characteristics that varied as a function of time. Specifically, based on this moving 3-component model, the following results were obtained. For SBP, the 1.0-year component reached statistical significance ($P<0.05$) in 157 of 167 (partly overlapping) intervals (94.0%). The 0.5-year and 0.41-year components were statistically significant in 110 (65.9%) and 39 (23.4%) intervals, respectively. In the case of DBP, the 1.0-year, 0.5-year, and 0.41-year components reached statistical significance in 152 (91.0%), 61 (36.5%), and 38 (22.8%) intervals, respectively, and in the case of HR, statistical significance was reached in 112 (67.1%), 61 (36.5%), and 37 (22.2%) intervals, respectively. Whereas in RBS (but not in FH

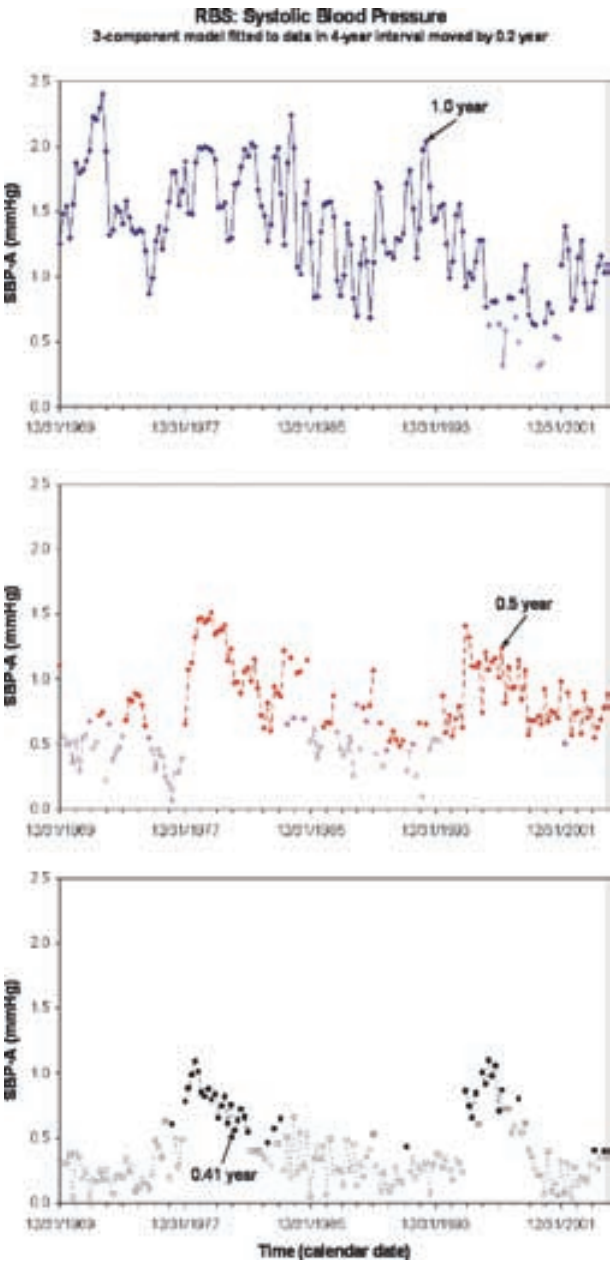


Figure 5
Statistical significance (P<0.05) of 1.0-year (top), 0.5-year (middle), and 0.41-year (cis-half-year, bottom) components fitted concomitantly to the SBP data of RBS is represented as dark-filled symbols, borderline statistical significance (0.05<P<0.10) as lightly-filled symbols, and non-significance (P>0.10) as open symbols. It can readily be seen that instances when the cis-half-year reaches statistical significance do not occur at random but mostly as clusters in time. Large changes in the amplitude of all three components are also observed for all three variables. © *Halberg*.

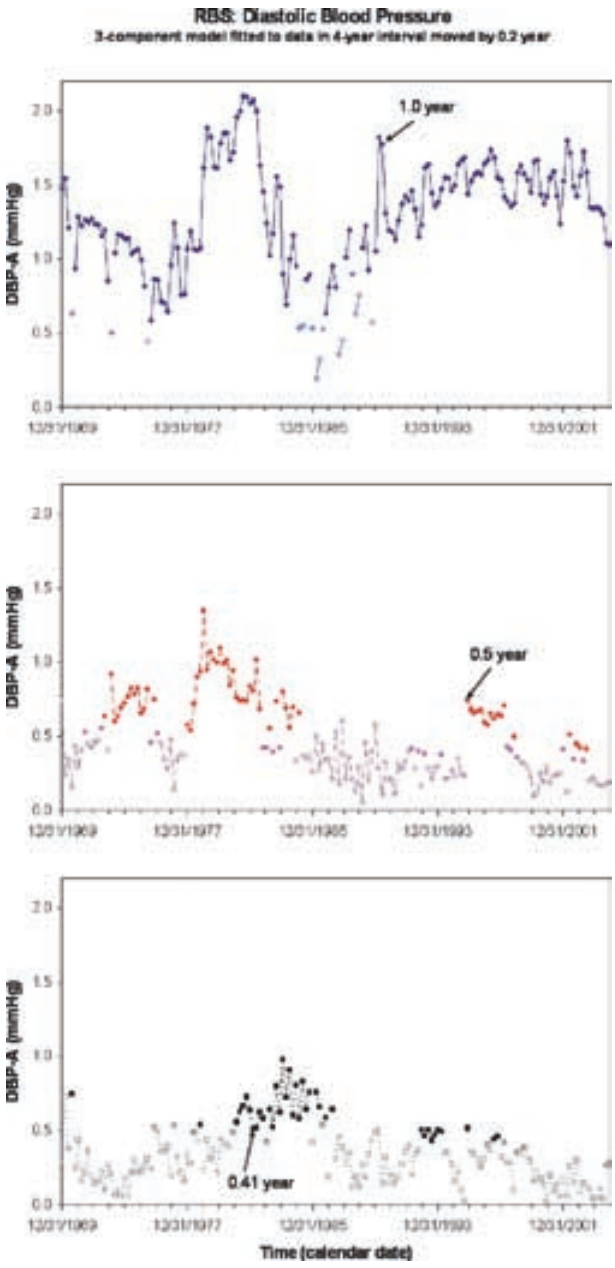


Figure 6
Statistical significance (P<0.05) of 1.0-year (top), 0.5-year (middle), and 0.41-year (cis-half-year, bottom) components fitted concomitantly to the DBP data of RBS is represented as dark-filled symbols, borderline statistical significance (0.05<P<0.10) as lightly-filled symbols, and non-significance (P>0.10) as open symbols. It can readily be seen that instances when the cis-half-year reaches statistical significance do not occur at random but mostly as clusters in time. Large changes in the amplitude of all three components are also observed for all three variables. © *Halberg*.

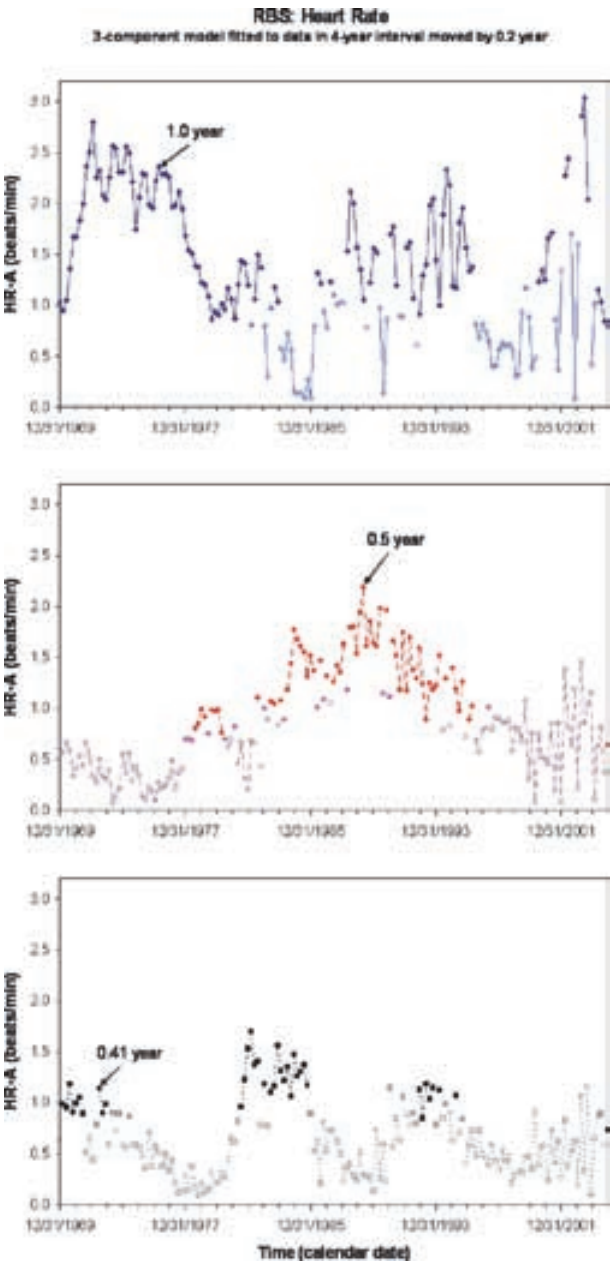


Figure 7
Statistical significance (P<0.05) of 1.0-year (top), 0.5-year (middle), and 0.41-year (cis-half-year, bottom) components fitted concomitantly to the HR data of RBS is represented as dark-filled symbols, borderline statistical significance (0.05<P<0.10) as lightly-filled symbols, and non-significance (P>0.10) as open symbols. It can readily be seen that instances when the cis-half-year reaches statistical significance do not occur at random but mostly as clusters in time. Large changes in the amplitude of all three components are also observed for all three variables. © *Halberg*.

and GSK), the yearly component and its second harmonic are undoubtedly the most consistent and the most prominent, the cis-half-year is also detected with statistical significance at a rate higher than would be expected by chance alone and according to a non-random pattern in time that prompts the search for a similar clustering in environmental variables (Figure 1, left, bottom). All three components could be detected concomitantly in 33 (19.8%), 18 (10.8%), and 10 (6.0%) intervals for SBP, DBP, and HR, respectively.

The results are illustrated in Figures 5–7, where statistical significance (P<0.05) is represented as dark-filled symbols, borderline statistical significance (0.05<P<0.10) as lightly-filled symbols, and non-significance (P>0.10) as open symbols. It can readily be seen that instances when the cis-half-year reaches statistical significance do not occur at random but mostly as clusters in time. Large changes in the amplitude of all three components are also observed for all three variables.

The occasional (intermittent) presence of all three components coexisting concomitantly in the BP and HR data of RBS was of interest in relation to another study in Florence, Italy, on circulating melatonin, offering a fresh interpretation to results obtained earlier. In the course of ~4 years, blood samples had been obtained at 4-hour intervals for 24 hours from each of 172 subjects for the determination of melatonin [24]. Original analyses of the serially-independent data pooled from all subjects stacked over an idealized year had reported the presence of a circannual variation characterizing daytime samples, contrasting with the detection of a half-yearly component in nighttime samples [24]. A recent reanalysis of these data without stacking revealed the presence of a cis-half-year instead of the originally reported half-year, Figure 8 [25].

PHASE BEHAVIOR OF THE YEAR, HALF-YEAR, AND CIS-HALF-YEAR COMPONENTS IN BP AND HR

Figures 9–11 illustrate the time course of the acrophases of the three components with periods of 1.0, 0.5, and 0.41 year, fitted concomitantly in ~4-year intervals displaced in ~0.21-year increments. The acrophases are shown only when the zero-amplitude test could be rejected at P<0.10. The yearly component is detected most of the time for SBP and DBP, but less often for HR. There are large changes in the circannual acrophases as a function of time, with a range larger than 4 and 5.5 months for SBP and DBP, respectively, and exceeding 7.5 months for HR, indicating that this component is much less stable than could be anticipated from the strong seasonal changes in the environmental temperature of Minnesota. The 1.0-year component may be modulated by lower-frequency components. The 0.5-year and 0.41-year components are only sporadically detected and their time course shows an

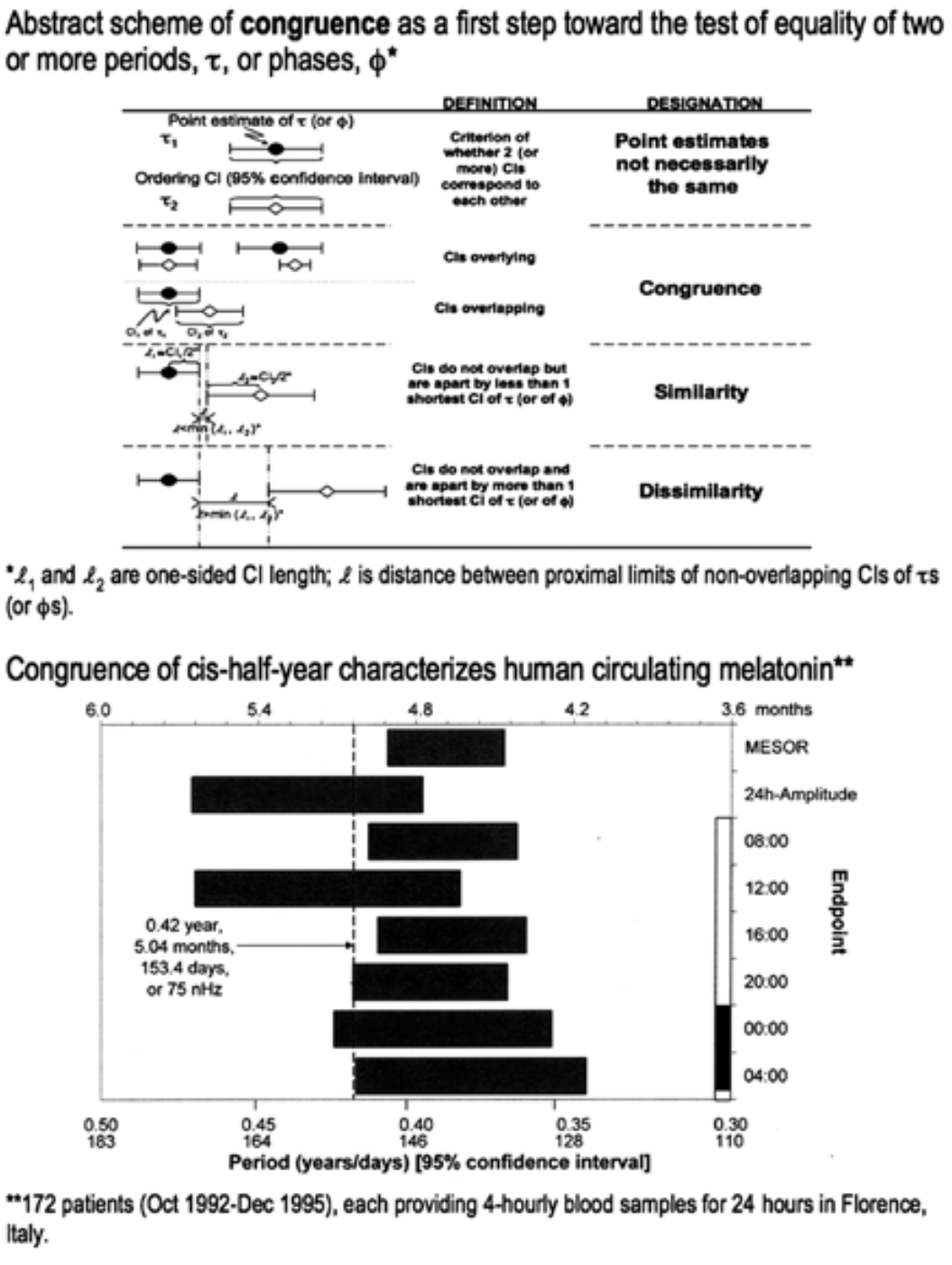


Figure 8
In melatonin data that were serially-dependent along the 24-hour scale, each person providing blood at 4-hour intervals for 24 hours, but serially-independent after pooling across 172 subjects examined in the course of ~4 years, a cis-half-year rather than half-year is invariably detected observed, characterizing the individual MESORs and circadian amplitudes as well as both daytime and nighttime samples considered separately. A map of the nonlinear estimates of cis-half-year periods and their CIs (bottom) is provided with an abstract definition of congruence (top). © *Halberg*.

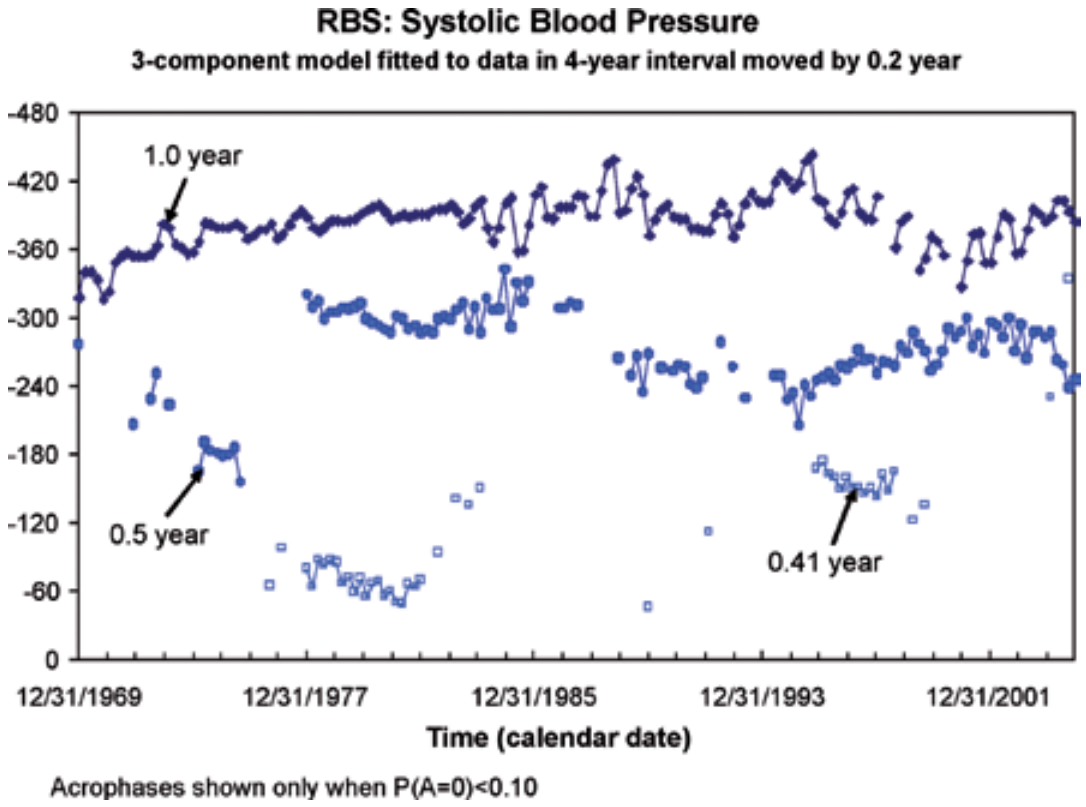


Figure 9
Time course of the acrophases of the three components (with periods of 1.0, 0.5, and 0.41 year) fitted concomitantly to the SBP data of RBS. Acrophases are shown only when the zero-amplitude test could be rejected at P<0.10. The 1.0-year component is detected most of the time, and all three components are occasionally detected concomitantly, albeit only intermittently. © *Halberg*.

intermittency contrasting with the relative consistency of the yearly component.

GLOBAL ANALYSES ASSESS ADDITIONAL COMPONENTS CHARACTERIZING BP AND HR

Apart from the cis-half-year, other nonphotic components characterize RBS's data. One of these components has an anticipated period of ~0.55 year, too close to 0.5 and 0.41 year to be reliably resolved in ~4-year intervals. Additional global analyses were hence performed on the entire 39-year series (1968–2006). Because some low-frequency components account for a much larger portion of the overall variance, analyses were processed in two steps. In the first step, components with periods longer than ~4 years were assessed nonlinearly together with a fixed yearly component and its second harmonic of 6 months. The model was then subtracted from the weekly means. In the second step, residuals were nonlinearly analyzed (allowing the period to vary as a parameter to be estimated) to try to resolve the remaining components of interest and to determine their (period) length. A composite model using the nonlinearly estimated periods was then fitted linearly to determine the extent of their statistical significance.

Low-frequency components considered had trial periods of 25, 11.1, 6.5, and 4 years. They were fitted concomitantly with fixed 1.0- and 0.5-year harmonically-related components. Nonlinear results indicated that all components could be resolved for SBP, the periods and their CIs being estimated as 22.56 [21.20, 23.92], 11.44 [10.92, 11.96], 6.66 [6.44, 6.88], and 4.03 [3.91, 4.15] years. In the case of DBP, all infra-annual components were also validated nonlinearly conservatively, whereas the half-year could only be assessed on the basis of a (more "liberal") 1-parameter CI of the amplitude that did not overlap zero. The period estimates for DBP were 20.55 [19.00, 22.10], 10.75 [10.14, 11.37], 6.70 [6.38, 7.03], and 3.96 [3.82, 4.10] years. In the case of HR as well, only the half-year had only a 1-parameter CI of the amplitude that did not overlap

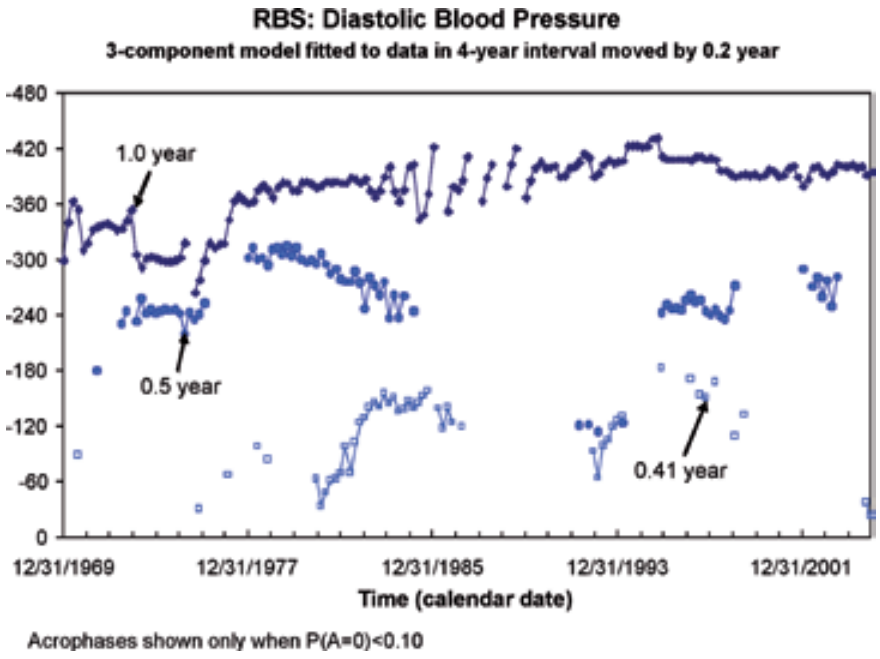


Figure 10
Time course of the acrophases of the three components (with periods of 1.0, 0.5, and 0.41 year) fitted concomitantly to the DBP data of RBS. Acrophases are shown only when the zero-amplitude test could be rejected at P<0.10. The 1.0-year component is detected most of the time, and all three components are occasionally detected concomitantly, albeit only intermittently. © *Halberg*.

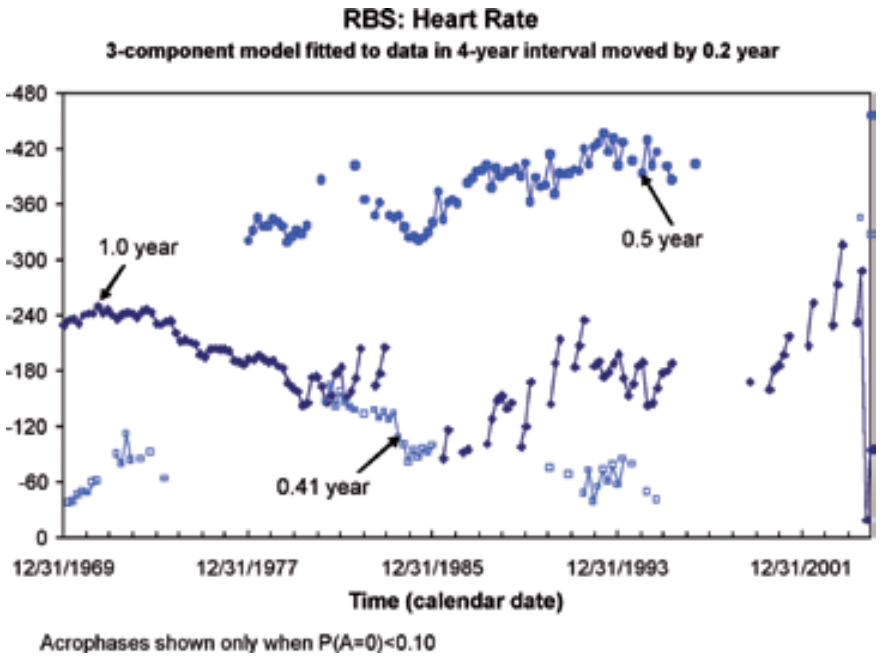


Figure 11
Time course of the acrophases of the three components (with periods of 1.0, 0.5, and 0.41 year) fitted concomitantly to the HR data of RBS. Acrophases are shown only when the zero-amplitude test could be rejected at P<0.10. The 1.0-year component detected most of the time in the case of SBP and DBP is less consistent for HR. All three components are occasionally detected concomitantly, albeit only intermittently. © *Halberg*.

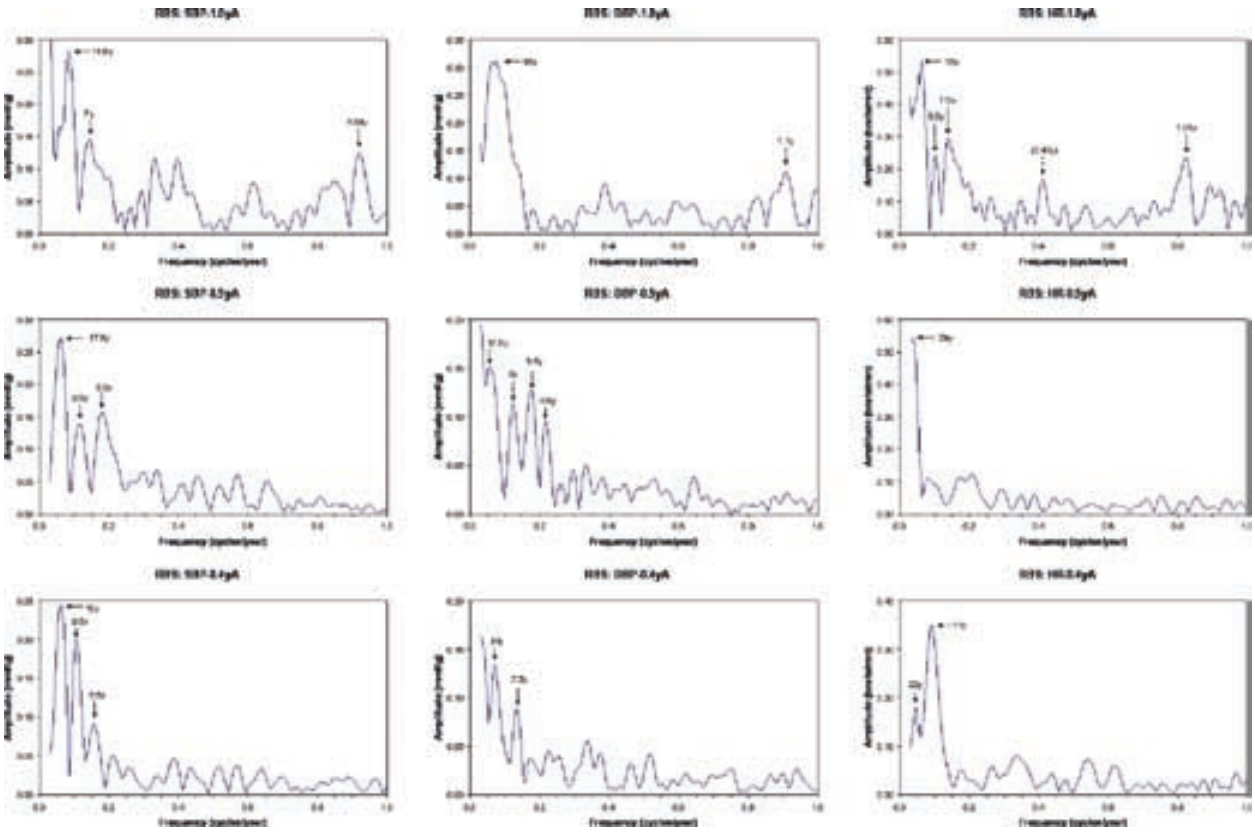


Figure 12
Least squares spectra of the amplitude estimates from the 3-component serial sections, with periods of 1.0-year (top), 0.5-year (middle), and 0.41-year (bottom) of SBP (left), DBP (middle), and HR (right) of RBS. P-values obtained in these analyses cannot be taken at their face value since the serial sections were pergressive with an increment of 1/19 the length of the interval. Perhaps because of the relatively large changes in amplitude and acrophase of the yearly component as a function of time, the 1.0-year amplitudes have an ~1.1-year spectral peak for SBP and DBP and by an ~1.2-year spectral peak for HR. The cis-half-year amplitudes of HR are characterized by a prominent ~11-year component. © *Halberg*.

zero. The period estimates for HR were 32.68, 13.59 [13.03, 14.15], 5.82 [5.71, 5.93], and 3.99 [3.91, 4.08] years. The selective multidecadal congruence is visualized in Figure 3.

Residuals from the above models indicated the presence of components with trial periods of 1.65, 1.38, 1.25, 0.55, and 0.41 year(s), corresponding to spectral peaklets in the global spectra of the weekly means. Nonlinearly, the three transyearly components were validated for SBP. Albeit the conservative approach yielded CIs of the amplitude that overlapped zero, the 1-parameter CI of the ~0.55-year and 0.41-year amplitudes did not overlap zero. With this qualification, period estimates and their CIs were 1.682 [1.646, 1.718], 1.400 [1.371, 1.428], 1.245 [1.226, 1.263], 0.548 [0.542, 0.555], and 0.413 [0.408, 0.417] year(s). In the case of DBP, the ~1.25-year component could not be validated and the other four components all had (if not conservative) 1-parameter CIs of the amplitude that did not overlap zero. With this qualification, the period estimates and their CIs were 1.695 [1.653, 1.738], 1.393 [1.339, 1.448], 1.235 [1.159, 1.310], 0.548 [0.537, 0.559], and 0.412 [0.407, 0.418] year(s). In the case of HR, a slightly different trial period had to be used for the first transyear: instead of a trial period of 1.65 years, one of 1.86 years was used. This component and the ~1.38-year component were both validated conservatively. The other three all had a 1-parameter (but not a conservative) CI of the amplitude that did not overlap zero. With this qualification, period estimates and their CIs were 1.806 [1.760, 1.851], 1.394 [1.371, 1.417], 1.247 [1.223, 1.271], 0.550 [0.541, 0.559], and 0.411 [0.408, 0.415] year(s).

A 5-component model using the periods estimated nonlinearly was then fitted, the periods differing slightly for each variable. This model and each of its constituent components

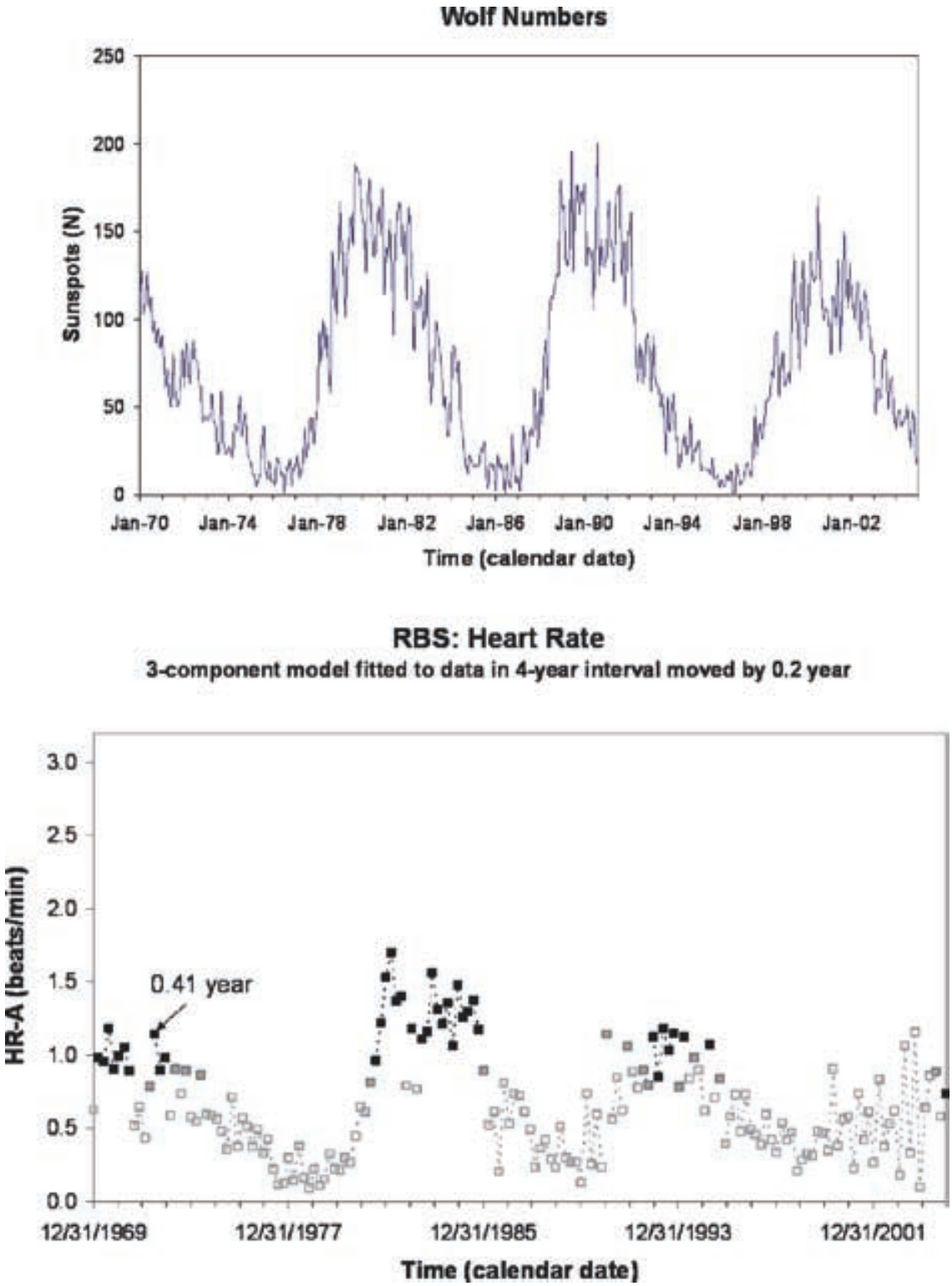
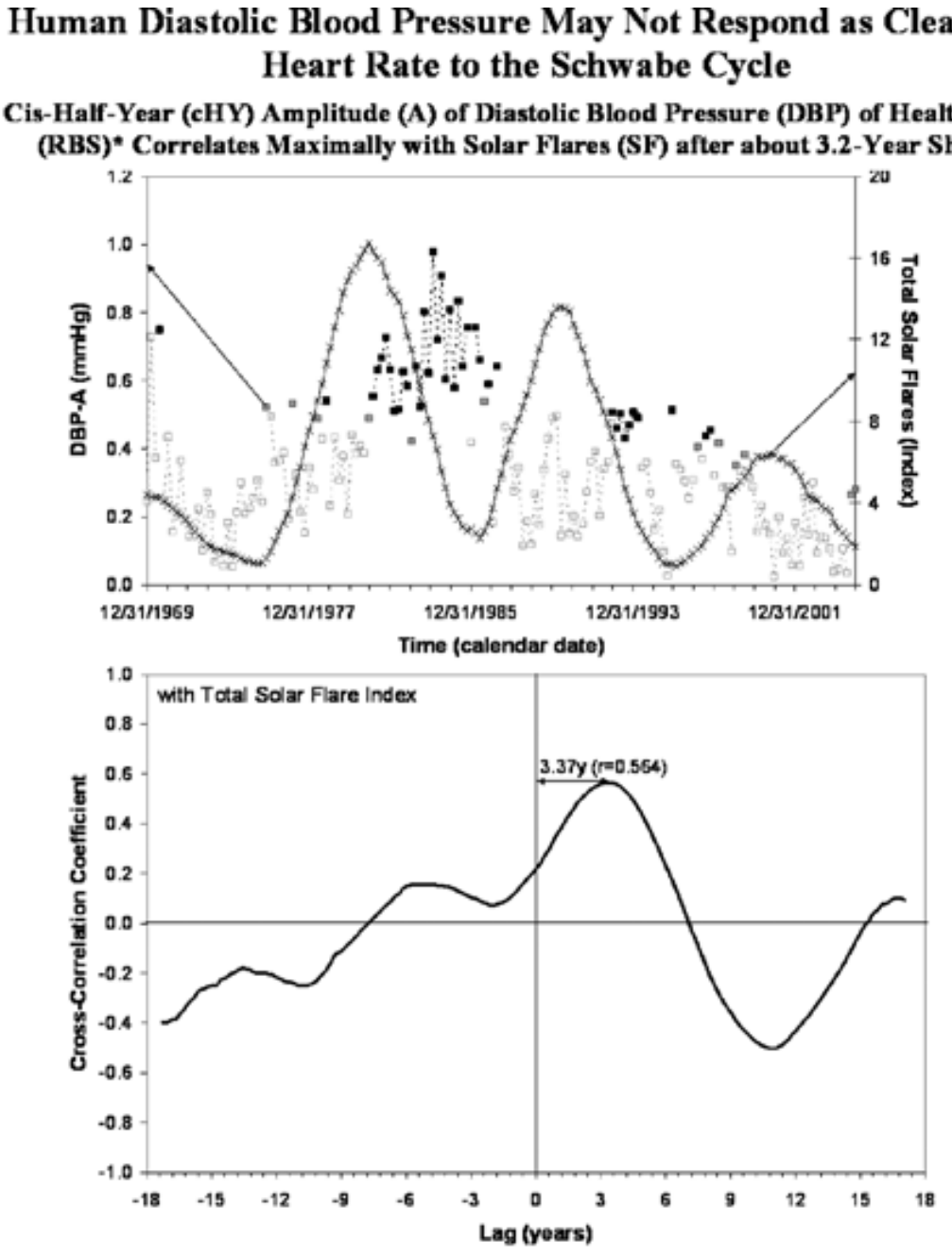


Figure 13
As also summarized in Figure 1, the cis-half-year amplitude of HR (bottom) shares an ~11-year cycle with Wolf numbers (top).
© *Halberg*.



* Assessed in 4-year intervals moved by 0.2-year over 39 years (1968-2006).

** Top: Time course of RBS' DBP-A(cHY) (squares) and SF Index (crosses). Data are weekly averages analyzed in 209-week intervals moved by 11 weeks. DBP-A(cHY) estimated by 3-component model consisting of cosine curves with periods of 1.0, 0.5, and 0.41 year(s). P-values from zero-amplitude test <0.05, 0.05<P<0.10, or P>0.10 are shown as solidly-filled, lightly-filled, or open squares, respectively. SF are 209-week moving averages, computed every 11 weeks, to match HR-A(cHY). Bottom: Cross-correlation coefficients of DBP-A(cHY) versus SF show a maximum at 3.37

Figure 14

Similarly to HR, the cis-half-year amplitudes of DBP are cross-correlated with solar flares, reaching a peak association of 0.564 at 3.37-year lag after the total solar flare index. © *Halberg*.

was statistically significant for SBP and for HR, but in the case of DBP, only the ~1.69-year and ~1.39-year components reached statistical significance, whereas the 0.41-year component only reached borderline statistical significance (P=0.054). The other two components with periods of ~1.24 and ~0.55 year(s) were not statistically significant and had to be removed from the model.

INFRA-ANNUAL MODULATIONS OF THE 1.0-YEAR, 0.5-YEAR, AND 0.41-YEAR AMPLITUDES OF BP AND HR

In order to explore possible environmental influences underlying the changes in amplitude of the 1.0-year, 0.5-year, and 0.41-year components of SBP, DBP, and HR, the amplitude estimates from the 3-component serial sections were further analyzed by least squares spectra, with the understanding that P-values obtained in the following analyses cannot be taken at their face value since the serial sections were pergressive with an increment of 1/19 the length of the interval. With this qualification, Figure 12 summarizes the results, rough estimates of the periods corresponding to spectral peaks marked in each case. Apart from some low-frequency components that may contribute sidelobes in global spectra of the weekly means, the following two observations may deserve further investigation. First, perhaps because of the relatively large changes in amplitude and acrophase of the yearly component as a function of time, the 1.0-year amplitudes are characterized by an ~1.1-year component for SBP and DBP and by an ~1.2-year component for HR. Second, the cis-half-year amplitudes of HR are characterized by a prominent ~11-year component.

Figure 13 (see also Figure 1) illustrates the ~11-year modulation of the cis-half-year amplitude of HR. Figure 14 shows that the cis-half-year amplitudes of DBP similarly lag after solar flares. RBS' HR and DBP as well as the total solar flare index are in synchrony with a decadal Horrebow-Schwabe cycle. As shown in Figure 2 (and discussed elsewhere), HR and Wolf numbers are also modulated by the transtridecadal **B**rückner-**E**geson-**L**ockyer (BEL) cycle (17, 26, 27).

Methodologically and basically revealing is that the search for another biotic cis-half-year has not only validated the latter in the longest available series of BP and HR, but has found near-transyears in SBP and DBP, whose presence had also been documented in physics (prompted by their presence in the elderly's BP). Whether the near-transyear is a nonlinear modulation by a lower frequency in physics, e.g., in solar magnetism or a component in its own right, it has a biological counterpart. Further, as to reciprocal periods of the beats of the rotations at different solar latitudes, Charles Wolff's predicted periods have been found not only as a cis-half-year but also as a trans-half-year of 0.55 year. At one end of the CI, its period is within one decimal of Wolff's 0.56-year prediction [23]. Moreover, both these periods have been validated along with a very likely geomagnetic component of 0.50 year.

CONCLUSION

Multiple spectral component signatures of time-varying solar activity are likely to characterize any time series covering years. When an anticipated component is not detected by the fit of a single component, a concomitant fit of several components is indicated. Apart from this methodologic truism that bears emphasis as the control information for any and all studies, we here demonstrate the intermittency of cis-half-years in the adult human circulation and their temporal association with an about 11-year cycle in solar flares, with a lag somewhat longer than 3 years.

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SUPPORT

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SYSTEMS PHYSIOLOGY AND CHRONOBIOLOGY AND THEIR RELATION TO MUSIC

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Dedicated to Franz Halberg on the occasion of his 90th birthday

ABSTRACT

In a short overview the following text presents a discussion of the meaning of time and of system-time in biology and medicine and points out certain time-dependent functions and additional conditions or items, all of which can be summarised under the title chronobiology and play an important role in physiology and pathology.

There is an intrinsic similarity between the systemic functions of a body and its subsystems, and the structure and function of music. Titles of publications like "Symphonie des Lebendigen" [1] and "symphony of life" [2] underline this metaphoric relation.

The essential components of a living system as well as of music are: time, certain structures or instruments which generate oscillations. These oscillations can be described by their frequencies, by the number of simultaneously generated frequencies, which may or may not be in harmonic relation to each other. The structure of music is supported by adjustment of the key, resonance and harmony, and by the larger time structures: rhythm and synchronisation, and oscillations and variability. These oscillations are subdivided by the given notation and by the defined tact, by prescribed tempo and by the interaction of different voices in terms of harmony, counterpoint, and rhythm. Periods and repeats characterise larger units, phrases, movements, pieces with different names and intention, or symphonies.

Including the expressions which were already mentioned, the following terms can be found applicable both in biological systems and in music:

Time
Cycles, Oscillations and Variability
Synchronisation
Evolution and Aging
Optimisation
Similarity and Scaling

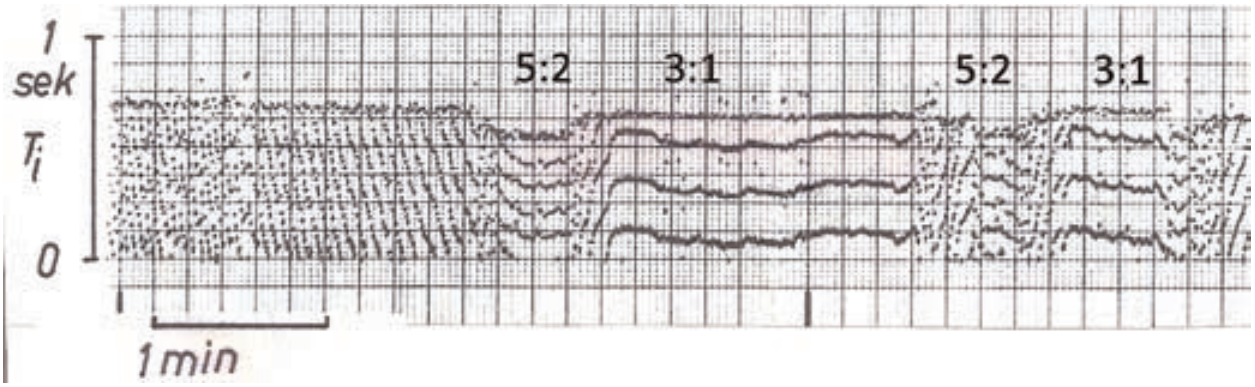

Figure 1
An example from an experiment in an anaesthetised rabbit, which was published in 1976 [8].
Ordinate: T_i duration of each breath.
Abscissa: time of recording.
As described in the text, during each breath the dots indicate a heartbeat. If heartbeat and respiration are synchronised, parallel lines are generated. The synchronisation 3:1 is easily recognised. The synchronisation 5:2 can be concluded from the fact that there was no change in heart rate during the whole time. The decrease of the distance of the lines indicates that two breaths are involved in the synchronisation (see text and Fig. 2 for detailed explanation).

Symmetry and Asymmetry
Chronification and Chaos
Sudden and Unexpected Phenomena

TIME AND SYSTEM-TIME

Life and time are essentially interdependent and can only be seen as an interacting complex. The recent consensus paper, which was initiated by Franz Halberg [3], signed in Brno and published in Moscow, shows the importance that chronobiology is attributed to within the present context of health and disease. It should be recognised that even the Latin word for time – *tempus* – can be attributed to ancient medical practice as will be described in the following.

In an article entitled "Rhythmen und Resonanzen" in a book on Viktor von Weizsäcker, Friedrich Cramer [4] writes the following summary on the meaning of time, starting with the interpretation of Newtonian understanding of time.

"The absolute true mathematical time flows smoothly as such and according to its nature, without any relation to external entities. This understanding of time of Newtonian physics is absolute, that means it is detached from external objects. This understanding was not given before. The Latin word *tempus* is derived from the region of the head, which has the same Latin name – temporal region. In the Hippocratic medicine the arterial pulse was palpated on the temporal artery. Time as *tempus* originally was our system time ("Eigenzeit"), the rhythm of our heartbeat, not detached from our personality. The new absolute meaning of time permitted to establish mathematical rules to describe movements and the paths of satellites. The Newtonian discoveries have enabled the technical epoch – even in medicine. However, this new point of view produced the effect that everything living was excluded from scientific research. Apparently, this is what Viktor von Weizsäcker meant when he wrote: *Until now, a moment in a biological process cannot be localized in the objective time, in the sense that a biological period cannot be measured by an objective clock…… This means that biological events and rhythms have to serve as a gauge for biological periods, whereas in physics time itself is the gauge for movement, velocity and accelerations*."

The distinction between physical and biological time, which appears as the essential statement in this text by Friedrich Cramer, can further be underlined by the following three observations:

At least in the time of Renaissance, musical notation was used to describe the "medical quality" of arterial pulses. Examples can be found in a respective book on music and medicine [5]. In the time of Renaissance there existed no metronomes. Therefore, the musician or the conductor of an ensemble had to use the time sequence of his own pulse to achieve an indicator of proper speed for a music performance.

Recently, several articles on medical aspects related to chronobiology have been published, whose titles refer to music. As mentioned above, a book by Friedrich Cramer has the title "Symphonie des Lebendigen" [1]. The list of similar titles also includes one article, recently published by Moser, Fruhwirth, Kenner, entitled "symphony of life" [2].

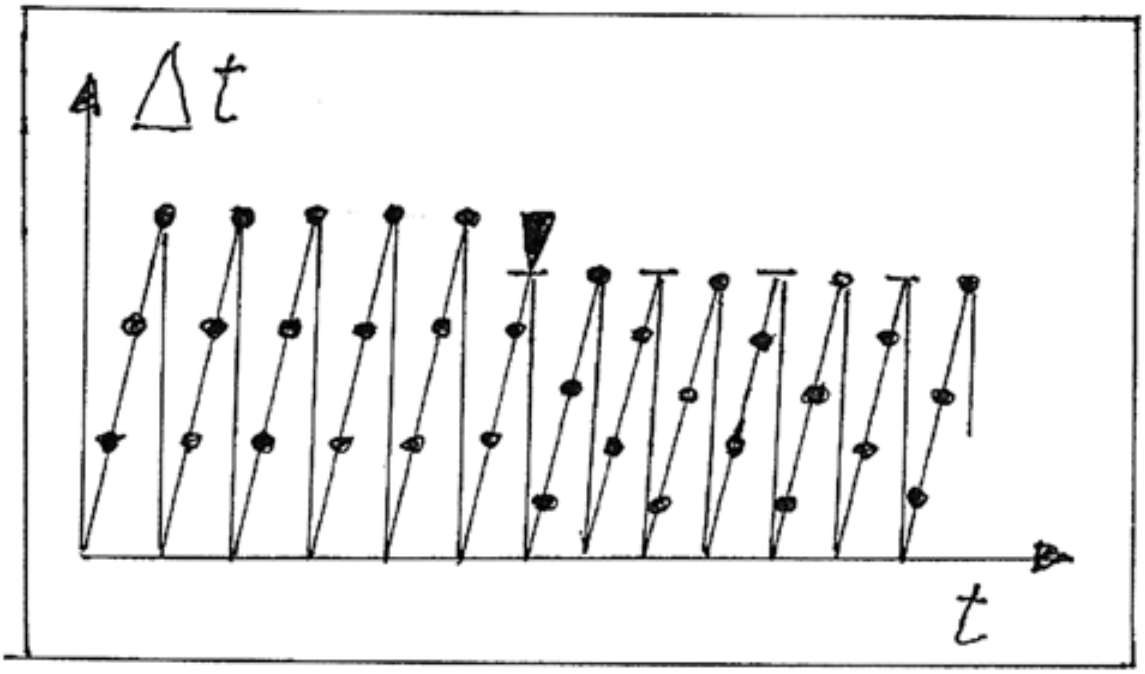

Figure 2
In this sketch the transition from synchronisation of breath duration to a heartbeat interval is demonstrated. Each breath starts at the zero value of the ordinate. The abscissa (t) represents time. Δt indicates the duration of breath. The dots indicate the time of the R-wave in the ECG. The vertical line indicates the reset of the process to "start of a new breath". The arrowhead indicates the change of synchronisation to 5:2, which is possible by reduction of the breath duration.

The term "system-time", which I used in the above translation of the German word "Eigenzeit", has been ever more in use. In 2002 a report on a symposium with the title "Selbstorganisierte Systemzeiten" (self-organised system times) was published [6].

CYCLES, AGING, OSCILLATIONS, AND VARIABILITY

Periodic or random oscillations and variability are characteristic features of most if not all biological variables.
On the one hand, time-dependent variations of biological variables may be generated or controlled by certain centres, like the autonomous circadian rhythm or the periodic heartbeat. On the other hand, there may exist periodic or non-periodic external influences acting on our body. In all these phenomena synchronisation of several oscillations may appear.
There is a list of characteristic time-dependent functions of life with respect to chronobiology. Most prominent are cycles and aging and, more generally, oscillations and variability. The description of the cyclic property of life and time can only be mentioned in some examples. The important role of cycles may be found particularly in ancient Egypt and also in Christian religion. The process of birth, growing, and aging can be found in circular pictures in churches, which describe life from birth to death and can be described as life-time clocks. One such picture from the 16th century can be found in the orthodox church in Arbanassi (Bulgaria).
Recognising the approaching end of his life, the Austrian Habsburg Emperor Karl V (1500–1558) had retired to the monastery San Yuste in Spain. He is reported to have tried to synchronise the many clocks which apparently existed in different locations in this building. Apparently this procedure did not succeed, a fact which he commented with the words: "clocks are as unreliable as humans". About a hundred years later, Christian Huyghens (1629–1695) discovered the phenomenon of synchronisation that he observed in two pendulum clocks, which were hanging on one common stick of wood.

Distinction must be made between normal controlled oscillations and oscillations which are due to some kind of instability. It can be assumed that any biological variable may, under certain conditions, achieve instability. Prominent examples are the loss of the control of the equilibrium of the body, and excessive "spontaneous" variations of blood pressure or blood glucose. As discussed in the consensus paper [3], variability of biological oscillations may be disturbed by abnormal amplitudes, or by shift of phases, or by irregularities, which generate unexpected outbreaks.

SYNCHRONISATION

It can be stated that more or less all oscillations tend to synchronise with each other [7]. The best-known example is the synchronisation of heartbeat and respiration [8]. A re-evaluation of the records published in our paper [8] revealed some more information about the detailed relation between heartbeat and respiration. One example is shown in Fig. 1. In our examples the rhythms can lock in phase-relations like e.g. 3:1, 4:1, 5:1; however, also 5:2. In order to explain this in detail, 3:1 means that during each respiratory cycle there are exactly 3 heartbeats. A synchronisation 5:2 means that two consecutive respiratory periods have the same length of time: the first of the two respiratory cycles contains a sequence of two and ½ cardiac cycles. The second respiration starts with the second ½ cardiac cycle and continues with further two cycles. During the synchronisation (5:2) this configuration is repeated as long as synchronisation is present. The transition of a rhythm from synchronisation 3:1 to the synchronisation 5:2 is shown in the sketch of Fig. 2. It should be mentioned that a more sophisticated analysis of the synchronisation relation can be found by the application of the so-called Hilbert transformation, as explained by Pikowsky et al. [7].

The appearance of these synchronisation phenomena has the closest similarity to certain music rhythms. The best-known rhythms are 3:1 and 4:1, which are also familiar from classical dance rhythms. A music tact 5:2 is less common, eventually characterising modern tunes. As far as the synchronisation of heartbeat and respiration is concerned, the relation 4:1 appears to be observable particularly in conditions of rest recovery and rehabilitation [9].

The consequence of an improved regularity during synchronisation of two biological systems appears to be an advantage for the co-operation of the two systems and an improvement of the transfer of energy or information.

EVOLUTION

Last year, in 2009, we remembered the 250th anniversary of the publication of Darwin's famous book "On the Origin of Species". His idea was that evolution was possible by variation and consecutive selection. As one example we can observe that the development of the cardiovascular system from a symmetric construction (fish) towards an asymmetric heart and an asymmetric aortic arch (mammals) apparently improves the efficiency of the system and the ability to adjust the function to changing load.

It can be seen that the following three further key words have to be involved in the considerations about evolution.

OPTIMISATION

Optimisation of function or of the use of mass or energy in a biological organism is as well a precondition as a consequence of the principle of evolution. The following example is chosen because of our special interest in the control of the cardiovascular system. In terms of the ventricular ejection one can show that the time course of ejection under the assumption of a certain given stroke volume and heart rate follows a path, which minimises the energy expenditure [10]. There is a more or less long list of examples which permit to demonstrate the natural search for optimisation of efficiency. Including the ability of adjustment to load, E. Weibel [11] has coined the name "Symmorphosis". This word expresses the importance of interaction between structure and function.

SIMILARITY AND SCALING

There exist between animals of different sizes the so-called allometric laws, which describe the relation between body mass, energy consumption, and other variables [12]. It is quite interesting and important that these laws also include what can be called individual system-time, and thus permit to illustrate what the term system-time means. The larger an animal the slower is system time passing. The allometric laws support the idea that structure and function of animals are optimally adjusted.

SYMMETRY AND ASYMMETRY

In physiology as well as in pathology and other fields of biology, the observation of the role of symmetry or asymmetry is of enormous interest. It was already mentioned above that, in the course of evolution, the asymmetric anatomy of the heart and the aorta served as a marked advantage for the adjustment of the cardiovascular system to variable load. There are, besides the heart and aorta, several organs which in mammals are markedly asymmetric. We have studied the effect of the asymmetry of aortic impedance, which is most probably involved in the improvement of arterial blood transport [13].

CHRONIFICATION AND CHAOS

There exists an interesting, rather simple equation, the so-called logistic equation, which is based on a feedback model where automatically the output of a calculation is fed back as the next input [14]. The logistic equation describes a kind of circular process. The result of such a calculation depends on one parameter. Depending on the value of this parameter, three characteristic types of resulting functions can be generated: 1. increase from zero to a limit level, 2. periodic oscillations, or 3. chaotic oscillations. Although this model is extremely simple, the processes of pathological variability or variability disorder or chronification and chaos can be explained.

SUDDEN AND UNEXPECTED PHENOMENA

Many diseases or pathological events happen completely unexpected. The explanation or discussion of such phenomena is the most difficult task. Many pathological events in medicine start unexpected, without warning. Examples are stroke, or heart attack. The so-called sudden and unexpected death of babies is another example of such an event. In the Austrian province of Styria we have developed a project for the prevention of sudden infant death [15]. The attempt to initiate such a project includes the search for possible risk factors and the education of parents. It can be shown that there exist internal risk factors – there is a higher incidence around 3 months of age – and external risk factors, like prone position or the smoking of parents.

SUMMARY

As initially stated, an attempt is made in this short text to list and shortly explain a – most probably incomplete – set of time-dependent biological (physiological or pathological) items, conditions, and functions. It can easily be seen that these items, functions or conditions are in a state of continuous interaction. The interaction and even entanglement of all of these functions have to be considered as one composite unit, like an orchestra, or the performance thereof, a symphony. In any case most or all of these items, functions, and conditions have to be taken into account if a special event in medicine or biology, e.g. a disease, is to be evaluated.

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BAROREFLEX OPEN-LOOP GAIN DURING 24 HOURS

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KEY WORDS

Blood pressure
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INTRODUCTION

During the second half of the twentieth century the opinion prevailed in the scientific community that the role of baroreflex in hypertension was negligible despite the fact that baroreflex is the most important regulatory mechanism of blood pressure. This opinion was supported by the observation of baroreflex resetting. The resetting is in other words a shift of the curve of the relationship between carotid sinus pressure and systemic arterial pressure to the higher values of systemic arterial pressure. This opinion was not shared by all scientists; the most prominent opponent, Professor Slight, claimed that it is clear that all forms of hypertension – whether primary or essential or secondary to renal, hormonal, or environmental influences – have a neurogenic component [1]. More recent evidence suggests that neural mechanisms, particularly impairment of arterial baroreflexes, play an important part.

In the recent years the long-term control of blood pressure was re-evaluated [2]. The recent opinion is based on chronic electrical stimulation of carotid baroreceptor afferent fibres, on re-evaluation of the time of chronic resetting lasting several days according to the more recent experiments. Furthermore, decreased baroreflex gain appears to precede hypertension.

Years ago, baroreflex sensitivity was regarded to correspond to the capability of the parasympathetic nervous system to react to a gross stimulus and thus to concern primarily vagal reflexes [3]. The measurement of baroreflex heart rate sensitivity in ms/mmHg was the adequate method. To study the blood pressure control function of baroreflex the determination of baroreflex gain is necessary.

The founder of modern chronobiology, Professor Franz Halberg, demonstrated many years ago that a reliable diagnosis of blood pressure disorders can be made on the basis of at least 24-hour-blood pressure monitoring. (Recent studies of Prof. Halberg suggest seven-day monitoring to obtain reliable estimates [4].)

The aim of the present paper was to determine the baroreflex open-loop gain during 24 hours in hypertensive patients and normotensive controls.

METHODS AND RESULTS

Blood pressure and heart rate were recorded in thirteen healthy subjects (N), mean age 33±12.3 years, all men, body height 178±8 cm, body weight 81±6kg, and in 14 patients with essential hypertension (EH), all men, mean age 40±6 years, body height 181±8 cm, body weight 83±8kg, in supine position, by means of Peňáz´s non-invasive blood pressure measurement. In EH medical tests and examinations excluded any other diseases and the diagnosis of essential hypertension was made according to WHO criteria to rule out any form of secondary hypertension. All patients had been followed up at least 3 years prior to the study at the department. All subjects gave their informed consent and the study was approved by the institutional ethical committee. Two inflatable cuffs were placed on both thighs. The cuff pressure was increased abruptly at 180 mmHg for 5 min. The occlusion elicited a vasodilatation of vessels in the legs. Following the abrupt change in pressure in the occluding cuffs from 180 to 60 mmHg caused a decrease in blood pressure followed by an increase of heart rate. The changes in the blood pressure and heart rate were used for calculation of the BRS. (The pressure of 60 mmHg in the occluding cuffs prevented stimulation of the low-pressure receptors by the increased venous return.) The mean systolic pressure and mean cardiac interval were from 5 beats preceding the abrupt decrease in the cuff pressure. The difference between the mean systolic pressure before and the minimum systolic pressure after the change in the cuff pressure was calculated. The difference between the mean cardiac interval before and the shortest interval after the change in the cuff pressure was also computed. The BRS corresponded to the ratio of these two differences and was expressed in ms/mmHg. The method is described elsewhere [5,6].

The BRS determination was repeated at 4-hour intervals during a 24-hour period. We started at 8 a.m. and finished at 8 a.m. on the next day. The daily activities of the subjects between the measurements were unchanged. The subjects were sleeping during the night but were awake during the measurements. The first measurement at 8 a.m. was not taken into account. Six measurements in each subject were used for further analysis.

The calculation of open-loop gain is based on the following equation. Baroreflex gain (G) corresponds to the decrease of blood pressure elicited by 1 mmHg increase of pressure in the carotid sinus:

$G = MBP\ (before) - MBP\ (after) = HR*SV*TPR - (HR-BRS_{hr})*(SV-BRS_{sv})*(TPR-BRS_{tpr})$, MBP – mean blood pressure, HR – heart rate, SV – stroke volume, TPR – total peripheral resistance, BRS_{hr} – heart rate baroreflex sensitivity [Hz/mmHg], BRS_{sv} – stroke volume baroreflex sensitivity[ml/mmHg], BRS_{tpr} – total peripheral resistance baroreflex sensitivity[(mmHg*s/ml)/mmHg = s/ml].

By dropping the second-order terms the equation can be simplified:

$G = G_{hr} + G_{sv} + G_{tpr} = MBP*(BRS_{hr}/HR + BRS_{sv}/SV + BRS/TPR)$,

G_{hr} – gain of heart loop, G_{sv} – gain of stroke volume loop, G_{tpr} – gain of total peripheral resistance loop.

Our method enables the calculation of BRS_{hr} and G_{hr} only. $G_{hr} = MBP*(BRS_{hr}/HR)$.

The results are seen in Table I.

Baroreflex measured as BRS (ms/mmHg) increases during the night as well as duration of the cardiac interval in normal controls; SBP and DBP decrease in both groups. The changes of baroreflex heart gain were not observed.

DISCUSSION

Our method did not enable the calculation of the gain of TPR and SV because SV and TPR were not measured in our experiments, but we suppose that the changes of all gains were similar. Nowadays we have the possibility of using a Task Force Monitor device for measurements in our laboratory. The estimation of the baroreflex set-point of MBF, which probably corresponds to the DBP night value, enables the calculation of the theoretical value of MBP without baroreflex and thus estimates the quantitative contribution of the baroreflex to the MBP value of a hypertensive subject.

Lower BRS values in hypertension were observed by Gribbin et al. many years ago by means of phenylephrine [7]. This finding was repeatedly confirmed by a non-invasive spectral method in our laboratory. This was explained in the past by the hypertension-elicited remodelling of the carotid arterial wall. The remodelling is a firmly established fact as indicated by the negative correlation between intima-media thickness and BRS [8, 9]. On the other hand, our calculation indicates that a lower baroreflex gain within the normal range of baroreflex in healthy people can make an important contribution to the blood pressure increase.

A second conclusion from our study indicates the necessity to re-evaluate the role of the blood pressure decrease during the night. Since the process of resetting lasts for about 48 hours, the night decrease of blood pressure influences the baroreflex resetting. The set point is surely lower than the night value and in subjects with normal blood pressure values during the night the shift of the carotid pressure-systemic pressure curve to higher values of pressure cannot occur. The normal chronobiology of blood pressure is thus a factor protecting against hypertension.

Table 1
Day and night differences in cardiovascular parameters in normotensives (N) and hypertensives (EH)

	N n = 10		EH n = 14	
	Day	night	day	night
I (ms)	921±181	1008±188	758±108	793±97
SBP (mmHg)	111±12	98±10	126±12	120±20
DBP (mmHg)	68±7	59±7	79±8	76±11
BRS (ms/mmHg)	11.64±4.95	20.90±21.80	8.42±6.52	8.35±5.34
G_{hr}	1.02±0.43	1.47±1.47	1.04±0.79	0.94±0.59

This is an additional argument supporting the view that the diagnosis and treatment of hypertension ignoring chronobiology are bad for the patient.

Healthy subjects (N), Patients with Essential Hypertension (EH), Cardiac Interval (I), Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), Baroreflex Heart Rate Sensitivity (BRS), Baroreflex Heart Rate Gain (G_{hr}).

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CIRCADIAN BLOOD PRESSURE VARIATION ANALYSED FROM 7-DAY AMBULATORY BLOOD PRESSURE MONITORING IN PATIENTS WITH ISCHAEMIC HEART DISEASE

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7 day blood pressure monitoring
Age healthy subjects
Patients after infarctus of myocardium

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INTRODUCTION

Franz Halberg, Germaine Cornélissen, and the BIOCOS scientific group provided strong evidence for the need to account for day-to-day changes in blood pressure and heart rate variables similarly as a circadian assessment considers the hour-to-hour variability [1–5]. The evidence led to the recommendation of around-the-clock monitoring for 7 days at the outset [6,7], to be continued whenever needed, until monitoring for a lifetime becomes more readily feasible.

By 1988, major findings had been summarised in a volume of annotated illustrations [8]. Methodology had developed concomitantly under Halberg Chronobiology Center leadership in Minnesota University. In particular, the "sphygmochron" [9] was introduced.

The sphygmochron is a computer summary of results from chronobiological analyses performed on BP and HR data collected around the clock by ambulatory monitoring. Two approaches are possible: 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 (**m**idline-**e**stimating **s**tatistic **o**f **r**hythm), 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.

The relationship between age and circadian blood pressure (BP) variation was described by us in 2004. One hundred and eighty-seven subjects (130 males, 57 females), 20–77 years old, were recruited for seven-day BP monitoring. Colin medical instruments (Komaki, Japan) were used for ambulatory

BP monitoring (oscillation method, 30-minute interval between measurements). A sinusoidal curve was fitted (least square method) and mean value and amplitude of the curve (the value of double amplitude shows approximately the day and night difference) were evaluated on every day of monitoring. The average 7-day values of the mean (MESOR) and of double amplitude (2A) for systolic BP (SBP), diastolic BP (DBP), and heart rate (HR) were determined for each subject. The 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. The 2A of SBP and DBP was increasing with age up to 35 years, then the curve remained relatively flat up to 55 years (maximum at 45 years), and then it decreased again (the difference between 45 and 77 years: SBP–13mmHg, DBP–12 mmHg). The heart rate M and 2A were age-independent.

The aim of the present study is the evaluation of blood pressure variability by 7-day ambulatory blood pressure (BP) monitoring in healthy subjects and in patients with ischaemic heart disease.

METHODS

The set being monitored consisted of 40 patients with ischaemic heart disease (IM) of the age 63 ± 6.3 years (age between 41 and 77 years) and ejection fraction (43 ± 12.3) %.

The patients were subjected to phase II of cardiovascular rehabilitation (controlled ambulatory rehabilitation programme) lasting for two to three months at a frequency of three times a week at the Department of Functional Diagnostics and Rehabilitation of St. Anne's Faculty Hospital in Brno. The duration of the training unit was 60 min and it consisted of a warm-up phase (10 min), an aerobic phase (25 min), a resistant training phase (15 min), and a relaxation phase (10 min).

In the course of rehabilitation the patients underwent 7-day ambulatory monitoring of blood pressure. During blood pressure recording they did not interrupt pharmacotherapy (ACE inhibitors, statins, beta-blockers, Ca antagonists).

The set of healthy subjects was composed of 44 healthy subjects (C, age between 40 and 77 years, mean age 54 years).

7-day monitoring of blood pressure was made by means of the instrument TM–2421 of the Japanese company AD operating on the principle of oscillometric analysis. The instrument measured blood pressure for 7 days repeatedly every 30 min from 5 to 22 o'clock and once an hour from 22 to 5 o'clock. If a value not much probable from the point of view of the instrument setting was recorded, another check measurement was made [9].

The results were processed by using Halberg cosinor analysis. The data were smoothed by a sinusoidal curve. The mean value of the sinusoid, designated MESOR, and the amplitude of circadian fluctuation were determined. A sinusoidal curve was fitted (least square method) and the mean value and amplitude of the curve (double amplitude corresponds to the night-day difference) were evaluated on every day of monitoring. The 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 for each subject of both sets.

The study was approved by the local ethical committee and the patients signed informed consent.

RESULTS

A significant increase of systolic BP (SBP) MESOR with age was found in C ($r=0.39$, $p<0.01$), but not in IM ($r=0.23$) (Fig. 1). Diastolic BP (DBP) MESOR was not related to age in C ($r=014$) but a decrease of DBP with age in IM was observed ($r=0.362$, $p<0.05$) (Fig. 2). The mean value of SBP MESOR was higher in C than in IM (128±9 vs. 121±8 mmHg, $p<0.01$), as well as DBP MESOR (81±7 vs. 74±7 mmHg, $p<0.01$). DA SBP decreased with age in C ($r=0.30$, $p<0.05$) but not in IM ($r=0.03$) (Fig. 4). Similarly, DA DBP decreased with age in C ($r=0.41$, $p<0.01$) and not in IM ($r=0.08$) (Fig. 5). Mean values of DA were lower in IM (DA SBP: 21±10 vs. 16±8 mmHg, $p<0.01$; DA DBP: 16±8 vs. 12±5 mmHg, $p<0.01$). Heart rate (HR) was not age-related in both groups, difference in mean values of HR was not observed (C: 71±10, IM: 65±8 bpm) (Fig. 3). DA HR was lower in IM (15±8 vs. 9±5 b.p.m) (Fig. 6).

DISCUSSION

There is a growing body of evidence suggesting that time structures in us and around us are intricately interwoven. Most if not all components of variation found in biota are also found in the environment, and vice versa [10]. For instance, about daily changes are seen in almost every biological variable under 24-hour synchronised conditions. It has also long been known that the phase of circadian rhythms can be manipulated by changing the phase of the environmental cycles [11]. At least for the case of circadian rhythms, their genetic inheritance has been demonstrated on a molecular basis [12,13], suggesting that the influence from the environment has been acquired genetically during the course of evolution.

The mapping of chronomes should benefit our understanding of human health and disease in several ways. The study of human chronomes can serve the derivation of refined reference values to better define health and to identify pre-disease, so that prophylactic interventions can be instituted as early as possible, preferably before the disease sets in [14–16]. The focus is thus put on pre-habilitation, in the hope that the need

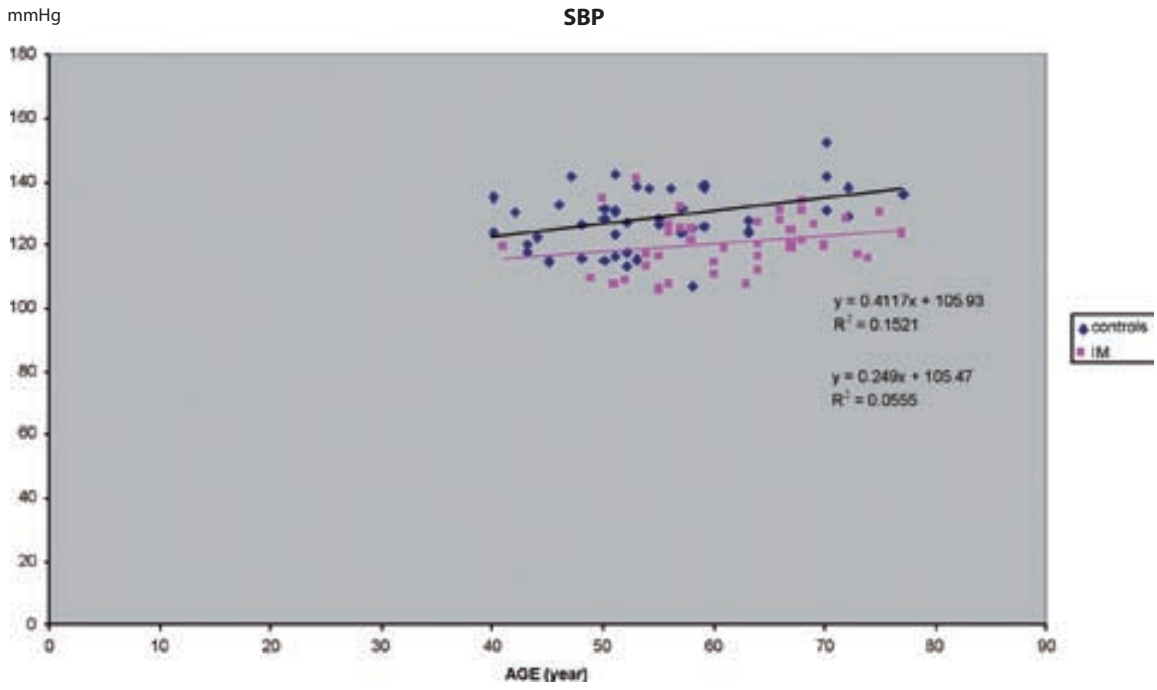

Figure 1
The relationship between MESOR of systolic blood pressure(SBP, mmHg), measured by 7-day ambulatory blood pressure monitoring, and age (years) of patients with MI and subjects C.

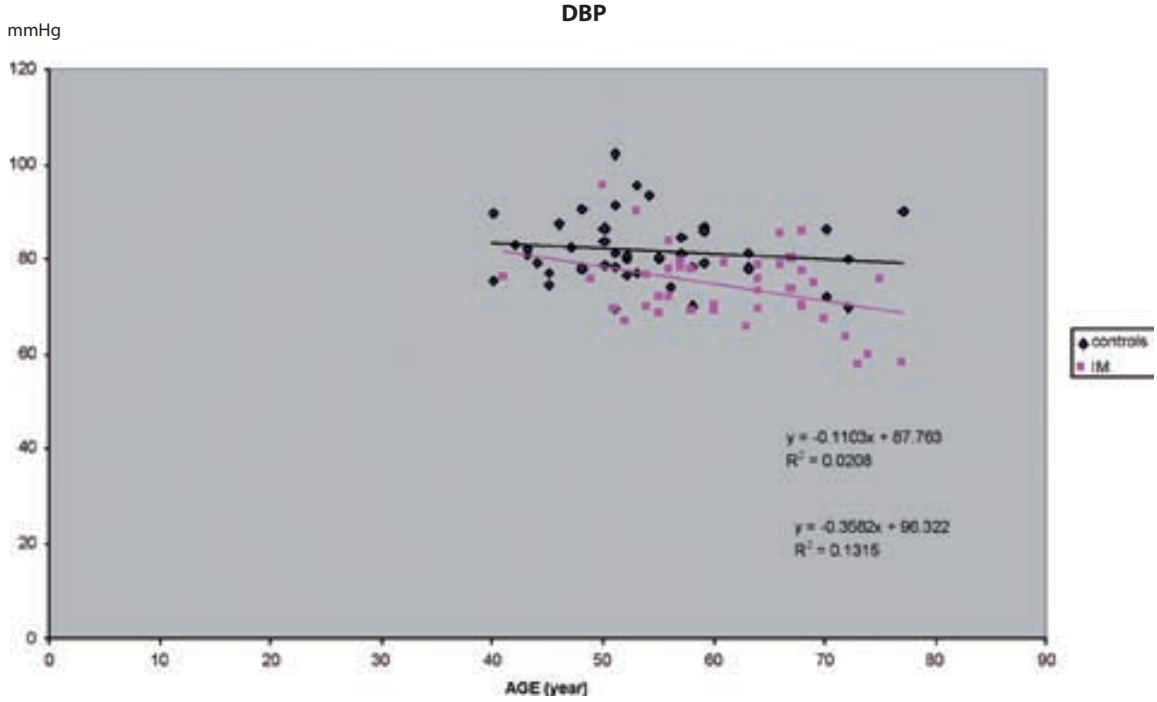

Figure 2
The relationship between MESOR of diastolic blood pressure (DBP, mmHg), measured by 7-day ambulatory blood pressure monitoring, and age (years) of MI and C subjects.

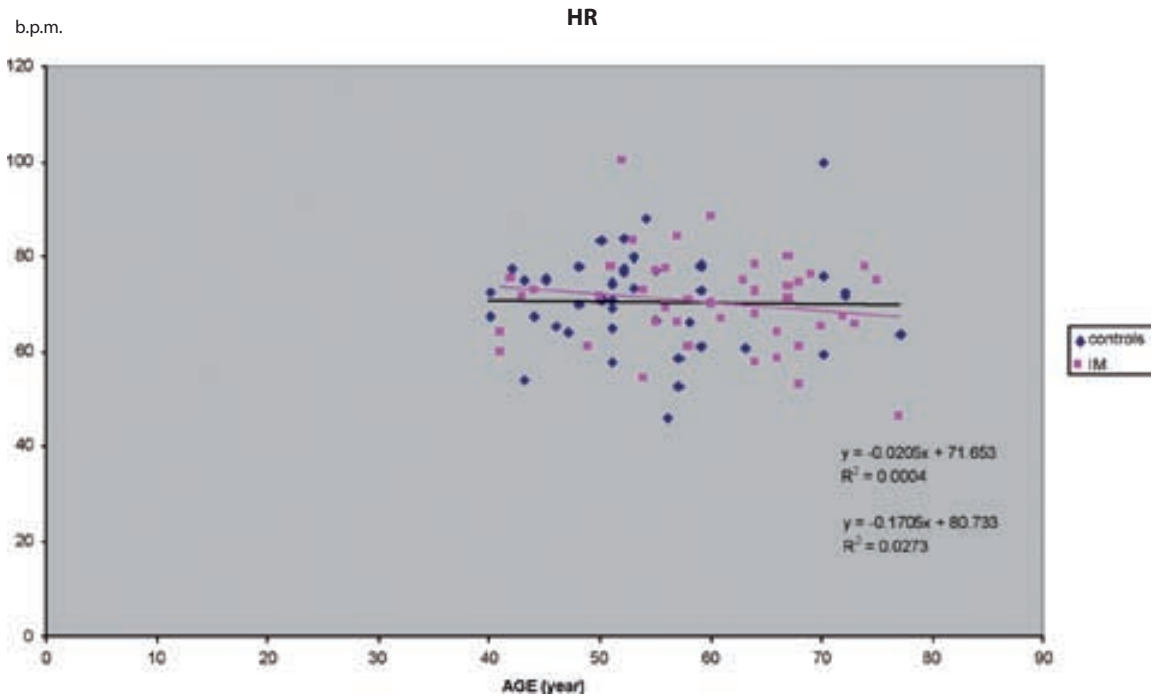

Figure 3
The relationship between MESOR of heart rate (HR, b.p.m), measured by 7-day ambulatory blood pressure monitoring, and age (years) of MI and C subjects.

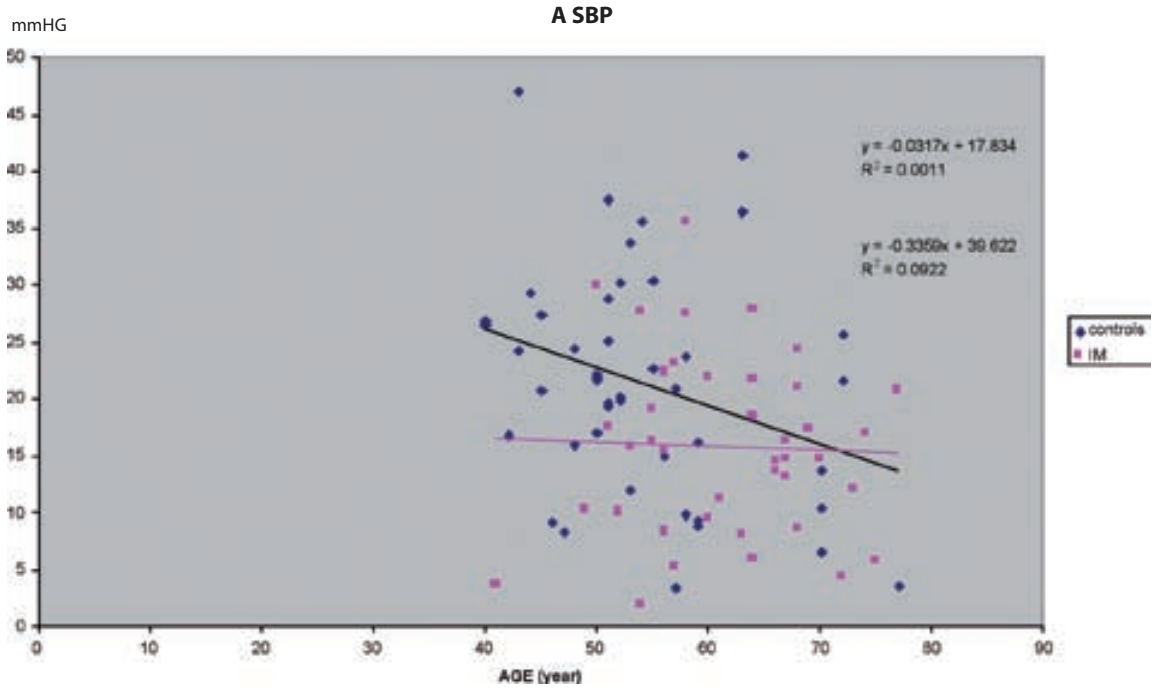

Figure 4
The relationship between circadian amplitude of systolic blood pressure (SBP, mmHg), measured by 7-day ambulatory blood pressure monitoring, and age (years) of MI and C subjects.

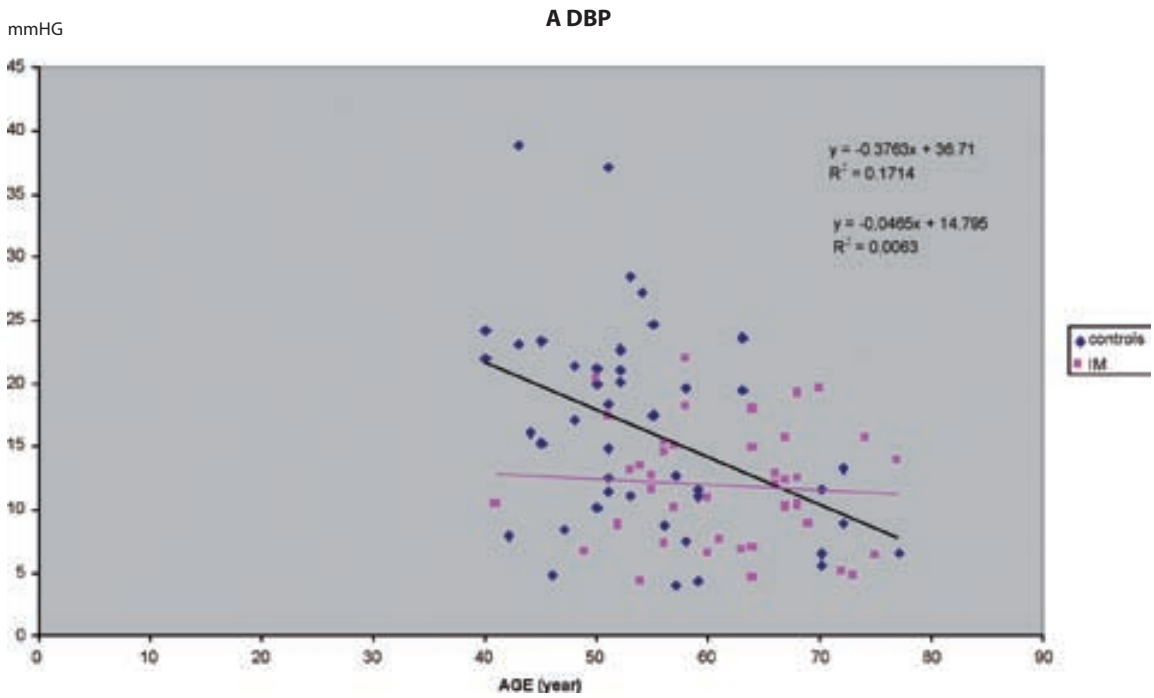

Figure 5
The relationship between circadian amplitude of diastolic blood pressure (DBP, mmHg), measured by 7-day ambulatory blood pressure monitoring, and age (years) of MI and C subjects.

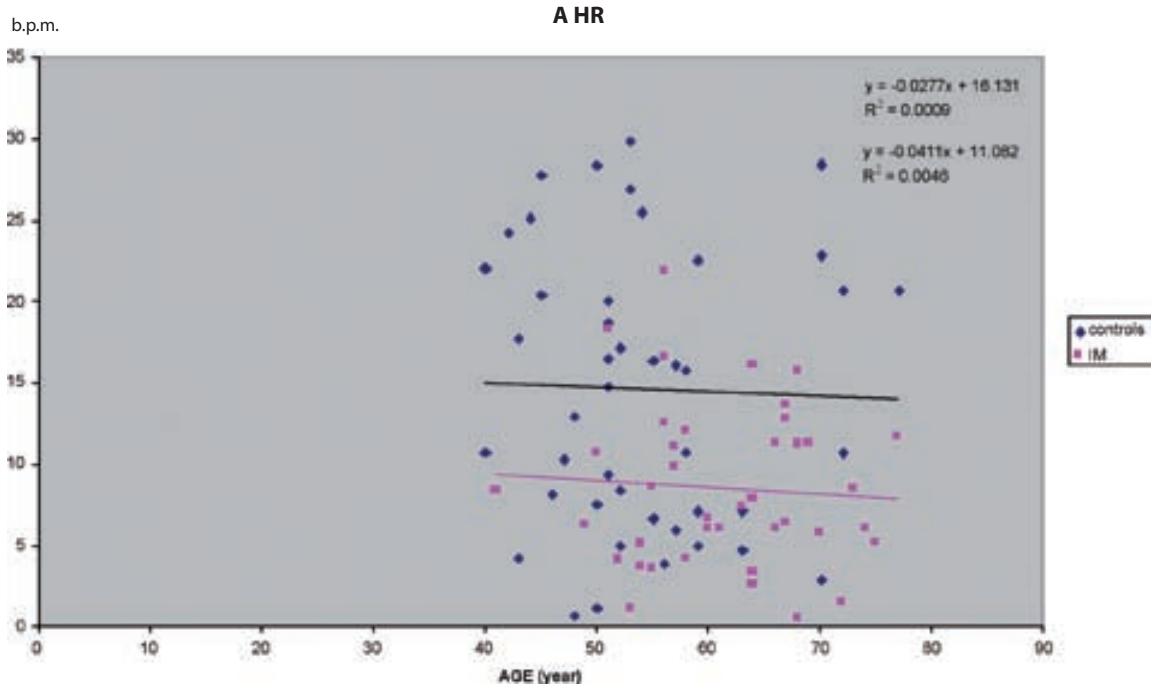

Figure 6
The relationship between circadian amplitude of heart rate (b.p.m, HR), measured by 7-day ambulatory blood pressure monitoring, and age (years) of MI and C subjects.

for re-habilitation will thereby be reduced [17,18,19].

Several studies [20,21] comparing the classification of patients based on single office measurements with that based on ambulatory monitoring for one to seven days suggest that the incidence of misdiagnosis is around 40 %, in keeping with the 48% response to placebo in the Australian Therapeutic Trial [22,23]. A 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 [24,25,26]. Prolonging the monitoring from one to two days reduces the uncertainty in the estimation of circadian parameters by about 35 % [27], whereas further information on the biological week [28,29,30,31] requires monitoring for at least 7 days, which is the current recommendation of BIOCOS for everybody at the outset [32]. It is now widely accepted that prognosis of target organ damage is by far superior when it is based on around the clock monitoring than on single office measurements [33,34,35].

The mistaken impression that the circadian variation in blood pressure and heart rate is sufficiently stable to be approximated by a single 24-hour profile stems in large part from the use of statistical methods on groups of subjects rather than focusing on the individual patient. Correlation analyses applied to large groups of subjects with a wide range of average values emphasise similarity. Statistical analyses focusing on individual differences observed from one profile to another, however, yield information more likely to help the patient in need of treatment [24]. Several case reports document this point [16,36,37,38,39]. Continued monitoring is the most logical solution.

An important distinction must be made between lessons learned from large clinical trials and their application for the individual patient. Differences and trends uncovered in studies made on groups, even when each subject provides only one or a few measurements, cannot be similarly assessed in medical practice when a decision must be made for treating the individual patient. In order to be able to reach an informed decision for the given patient, serial rather than single data should be collected. When time series are available, it becomes possible to assess risk elevation or the response to treatment for that particular patient.

In our former study we observed the age dependence of the circadian amplitude. Mean values of SBP and DBP were increasing with age up to 75 years, but the night-day difference of SBP and DBP reached the maximum value at 45 years and then decreased. This decline of the day-night difference was not seen in our patients with heart disease. Furthermore, the day-night difference in about 50-year-old treated patients was lower than in our about 50 years of age controls. This fact is positive because an excessive day-night difference is accompanied by an increased risk for morbidity and mortality.

A comparison of treated patients after myocardial infarction with healthy subjects revealed that the treatment decreased the risk of high blood pressure as well as the risk of high blood pressure variability. The differences could be influenced by medical therapy.

SUPPORT

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ANALYSIS OF TRACE ELEMENTS IN THE TEETH OF INDIVIDUALS FROM THE FORMER CRYPT IN ST. CATHERINE MONASTERY IN DECHTICE (DISTRICT TRNAVA, SLOVAKIA)

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ABSTRACT

The present article provides results of analyses of trace elements from dental tissues and their relations with social status, dietary habits, and pathological changes in vertebras in the skeletal remains from the former family crypt in the St. Catherine Church ruins. Three aristocratic families (the Labsánszkys from the Korlátko castle, the Erdődys and the Apponyis) were buried under St. Catherine church in the 18th century. Skeletal material from one of the three crypts was investigated. The family allegiance of these skeletal remains is still not known; our considerations based on the historical sources indicated the Labsánszky family. The concentrations of Ca, Sr and Zn in 8 permanent teeth obtained from 8 individuals were analysed. The number of analysed teeth was limited by the number of buried individuals and the preservation state of the skulls. Concentrations of the trace elements and their ratios – a relatively low content of strontium and a higher concentration of zinc – indicated a rich protein diet. Despite the small number of teeth analysed, the results are relatively homogenous and show that probably all of the buried persons had belonged to a higher society. The results were also confirmed indirectly by the palaeopathological findings in the bones of the postcranial skeleton. The Forestier disease (DISH) was diagnosed in three individuals at minimum, which can also indicate that they suffered from obesity or type 2 diabetes. However, DISH is a hereditary disease; therefore we must also consider the familiar appearance.

INTRODUCTION

Analyses of trace elements and stable isotopes have become a very useful research tool in physical anthropology for the

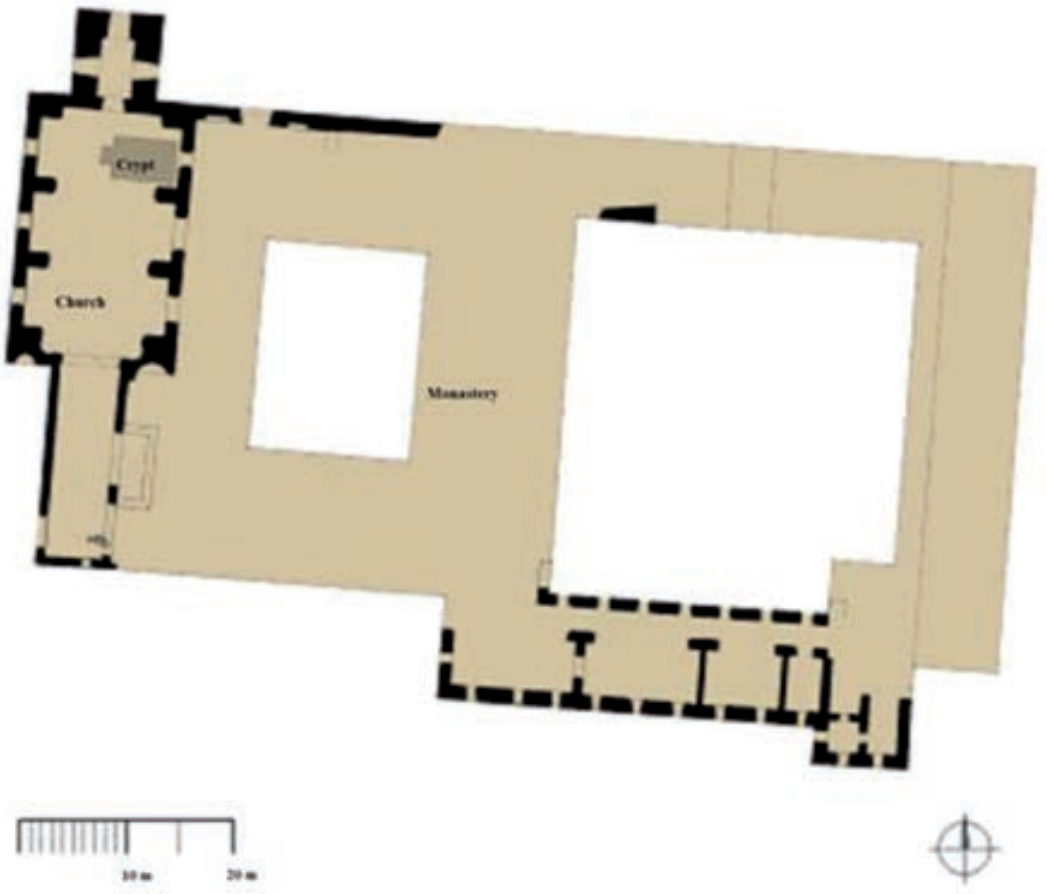

Figure 1
The ground plan of the crypt

last three decades. They offer the possibility of studying the biological condition of human groups [1, 2, 3, 4], dietary habits of past populations [5, 6, 7, 8], processes of diagenesis [9, 10], as well as the aetiology of some diseases [11]. The trace element analyses are also used for studying the type of nutrition in connection with the socioeconomic position of individuals or whole population groups [12].

In palaeoanthropological research focused on diet reconstruction a wide spectrum of macro- and microelements has usually been analysed. Strontium, zinc, calcium, barium, copper, and iron provide the best information about the biological status of the studied populations [6, 12].

The reliability of zinc concentration for diet reconstruction has been widely discussed [13]. Some authors [8, 13] proposed that the relation between zinc concentration and the type of nutrition is disputable. On the other hand, the existence of a positive correlation between the amount of accumulated zinc and protein diet has been confirmed [14, 15]. However, strontium absorption and accumulation is in inverse proportion to an organism's position in the trophic pyramid. Plants absorb and accumulate strontium directly from the environment, while mammals accumulate it indirectly, by consumption of plants and other animals [7, 8, 11, 16, 17]. Some authors indicate that strontium and Sr/Ca ratio do not provide direct information on diet components and cannot be used as a straight indicator of the trophic position of organisms [8, 18].

However, in our study we tried to reconstruct the quality of alimentation based on the mentioned trace elements and to find relations to Forestier disease (DISH) and the aristocratic origin of individuals from the former family crypt of the St. Catherine church.

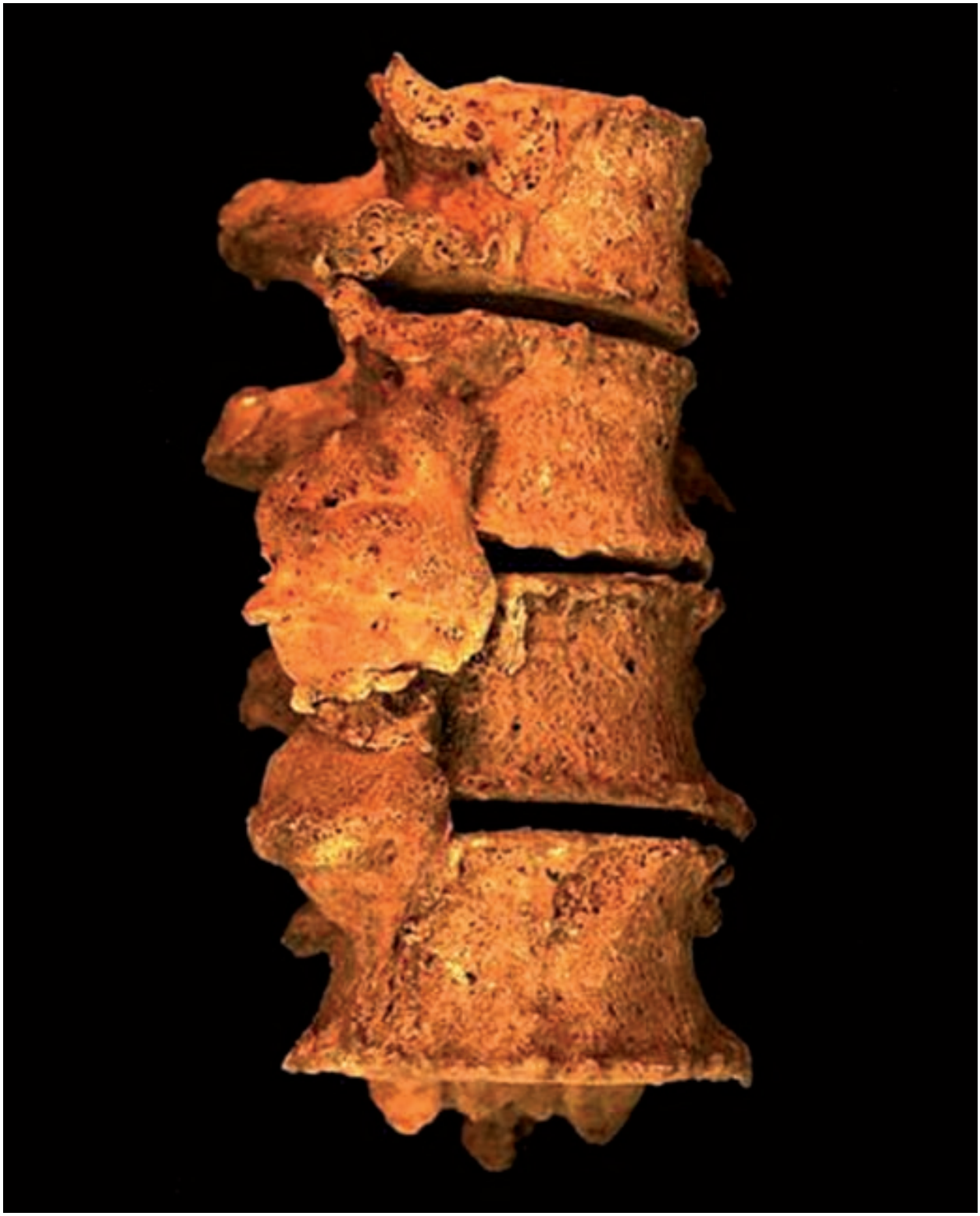

Figure 2
The 9th -12th thoracic vertebras with suspected DISH

HISTORICAL AND ARCHAEOLOGICAL CONTEXT

The monastic complex of St. Catherine is situated in Western Slovakia, around 20 kilometres north of Trnava, close to the villages of Dechtice and Naháč.

The beginning of the monastery dates back to the end of 16th century, when St. Catherine appeared reputedly several times in this place. The cloister was built in 1618 by Count Christopher Erdődy for 12 Franciscan monks. Soon after the foundation the monastery was demolished and a larger one was built on its place. After rebuilding, which Count Erdődy's son Gabriel with his wife had finished, the church occupied rather a large area. There were eight altars at the time of dedication. Three familial vaults were also situated in the church, in which members of three aristocratic families (Erdődy, Labsánszky, and Apponyi from Korlátko) were buried.

In the 17th century, the monastery was on several occasions close to ruination. The monastery and church were stripped and burned by the armies of Juraj Rákóczi during the first rebellion. Later Turks hijacked the monastery and finally soldiers of Imre Thököly sacked the complex.

The monastery was closed on 22nd July 1786 by the order of Joseph II. After evacuation of the monks, the building was occupied by the Trnava's soldiers with army disabilities. After the closure of the monastery the vaults were robbed on many occasions. According to written sources, the first forced entry into aristocratic vaults was carried out in 1793. Count Joseph Erdődy, out of respect to the remains of his forefathers, purchased the desolate objects in 1797. However, he was unable to prevent the monastery from falling into ruins.

Further raids were made in the 19th century, when the inhabitants of the surrounding villages, who disassembled the buildings for use as building material, systematically looted the church and monastery. The temple floor and the crypt arch were also dismantled within this period. Some bones were bundled out of the coffins and later repeatedly placed in the crypt together with building waste.

In 1995, the volunteers and devotees began with conservation of the ruins. Archeological examination of this locality started in 1997 and its goals are to verify the written history of the monastery [19].

In 2000 and 2001, a part of the circumferential stall of the monastic building and of the crypt was revealed. Archeological examination in the church proceeded with geophysical investigation, specialised in searching for crypts and other cavities. As a result of this investigation, 3–4 places indicating the presence of cavities were located, while a marked anomaly was registered to the left side near the entrance [20, 21]. According to the canonical visitation of 1782, one of the three aristocratic crypts should be present here. A probe in this area uncovered a partially destroyed and disarranged crypt.

The crypt was built of brick; its inside had a rectangular shape with the dimensions of 350 cm x 190 cm and the end of the first third it was divided by a partition (*Figure 1*) [22]. The rolling vault finishing narrowly under the temple floor initially vaulted the crypt. All filling layers yielded skeletal remains mixed with building waste and stones. Only one incomplete skeleton in anatomical position was found in the crypt bottom. From the coffins, only a few fragments of wood ornamented with hemispherical rivets were preserved. Copperplate, initially gold-plated, rivets were organised into letters, but the bad condition of disintegrated wood did not allow reconstruction of the text.

Although the remains were found in non-anatomical positions, we have supposed for the last 8 years that they had belonged exclusively to members of the Labsánszky family. Furthermore, the families of Erdődy and Apponyi had their own crypt, and there are records regarding removal of the remains from the church. If our assumptions are right will probably show the opening of the two remaining crypts in the next years.

MATERIAL AND METHODS

Anthropological and palaeopathological analysis

As the bones were mixed with the filling, craniums and postcranial bones were individually examined. A departure from this was one individual torso (pelvis and lower limb bones) lying in an anatomical position in the crypt bottom [23].

After counting the long bones, namely the number of humeri, it was determined that there were remains of at least 26 individuals present. The number of skulls was lower; however, it was possible to differentiate 24 skulls in different stages of preservation. It was not possible to allocate some of the fragments to any of the skull, thus it is probable that the number of craniums in the crypt was higher. The distribution of the skulls based on age and gender is shown in *Table 1*, the number of postcranial bones with regard to the laterality is in *Table 2*.

It is also very difficult to estimate the number of individuals actually buried in the crypt. According to the archaeologist, the crypt is rather small for storing more than 20 individuals. It is possible that some of the skeletal remains came secondarily from another crypt in the times of raids.

The skeletal material was examined using standard morphoscopic and morphometric methods [24, 25]. Gender and age were determined only in the craniological material using the methods of Acsádi and Nemeskéri [26] and Lovejoy [27].

We also observed developmental defects, pathological changes, and traumatic lesions in all the examined remains.

Table 1
Distribution of the skulls based on age (in years) and gender

Age	Juv	Ad	Ad II-Mat I	Mat	Mat II-Sen	Sen	Indeterminate	Total
Sex	(16–20)	(20–40)	(30–50)	(40–60)	(50–60)	(60+)		
Male		2	3	1	1			7
Probably male	1			2	1			4
Female	2	1			1			4
Probably female					2			2
Indeterminate		3				1	3	7
Total	3	6	3	3	5	1	3	24

Table 2
The number of postcranial bones with regard to laterality

	Dexter	Sinister	Indeterminate	Total
Clavicle	17	10	1	28
Scapula	17	17		34
Humerus	26	26		52
Ulna	24	16		40
Radius	17	19		36
Sacrum	19	x	x	19
Os coxae	20	19		39
Femur	23	23		46
Tibia	23	21	3	47
Fibula	12	11	2	25

Laboratory methods

The concentrations of trace elements in 8 permanent teeth obtained from 8 individuals were analysed (*Table 3*). The number of analysed teeth was limited by the preservation state of the odontological material as well as by the relatively low number of skulls. In order to minimise the effects of diagenesis, which is frequent in bones, the analysis was restricted to teeth [7]. All analysed teeth were well-preserved, intact, without dental caries and abrasion.

Before the analysis, each tooth was washed with distilled water and dried at room temperature. Then the teeth were crushed in an agate mortar and homogenised. Altogether, 0.5 g of each sample was subjected to wet mineralisation in a mixture of 10 ml HF, 1 ml HNO_3, and 1 ml $HClO_4$. On the next day, the samples were evaporated in a water bath to approximately 1 ml volume. Then, the evaporation on a sand bath with a gradual addition of HF, HNO_3 and $HClO_4$ continued until the escape of dense smoke. Finally, 5 ml of saturated solution of H_3BO_3, 1 ml HNO_3 and $HClO_4$ was added and 1 ml of the samples was evaporated on a sand bath until dry. The dry residues were diluted with redistilled water, warmed in a water bath and, after the addition of 5 ml HNO_3, heated to a temperature of about 150 °C on a sand bath for 5 minutes. After cooling, the samples were transferred into 50 ml measuring flasks and diluted with spectrally pure water.

Blind tests were prepared parallel to the sample preparation to determine the analytical background. Analyses were made by optical emission spectrometry with inductively coupled plasma using the spectrometer ICP OES Jobin Yvon 70 Plus (France).

The contents of Ca, Zn, and Sr were examined. Two soil samples obtained during excavation of the grave were also subjected to analysis, the pH value and the same elemental concentrations were determined.

Statistical analysis

Data analysis was performed in R [28]. We used the Kolmogorov – Smirnov Goodness-of-Fit test for testing data normality (for all of the variables we did not reject null hypothesis about normality – all p-values were greater than 0.8). One sample Student t-test was used for testing if the content of trace elements in the teeth could be affected by diagenetic processes in the soil. T-test for zero linear correlation (measured by the Pearson product-moment correlation coefficient) was used to test association between the variables (Ca-Zn, Zn-Sr, and Sr-Ca). For all statistical tests, the significance level α was equal to 0.05.

RESULTS AND DISCUSSION

The pH values of the soil from the crypt were 8.66, or 8.91. The concentrations of the examined elements determined in the soil are listed in *Table 4*; descriptive statistics of the concentrations of elements in human teeth are shown in *Table 5*. Linear association between the variables Ca-Zn, Zn-Sr, and Sr-Ca was not statistically significant (all p-values were greater than 0.2).

Due to the small number of soil samples, we tested the equality of the mean value of each parameter in teeth (Ca, Sr, and Zn) with the representative soil samples for determination of possible diagenetic processes (*Table 6*).

According to the concentration gradient theory, ions from higher concentration areas tend to move to sites of lower concentration [8]. We did not find any differences between the concentrations of Sr in teeth and soil samples. On the other hand, significant differences were found in the Ca and Zn concentrations between teeth and soil. However, in the case of strontium diagenetic processes cannot be excluded, concentrations of calcium and zinc were not affected by diagenesis and indicate that ionic movement from soil to teeth would not be expected.

Since the buried individuals probably belonged to members of an aristocratic family, we supposed that their diet had been abundant in animal proteins. The results of the trace element analysis as well as the ratios of examined elements confirmed our assumptions; a relatively low content of strontium and a higher concentration of zinc expressly indicated a rich protein diet (*Table 5*). In spite of a small sample of teeth, the results are relatively homogenous and indicate that all of the buried persons could belong to a higher society. Similar results were found by Prokeš and Hegrová [29]. Chemical analysis of the skeletal remains of Dietrichstein family members showed that they had consumed food rich in meat and pastry, and poor in milk, fruit, and vegetables.

Table 3
List of analysed skulls and teeth

Skull	Gender	Age (in years)	Analysed tooth (FDI)
K01	M	30–40	17
K03	M	30–50	35
K04	M	30–50	45
K05	NA	20–40	28
K07	M	20–30	18
K09	M	45+	15
K12	M	30–50	48
K13	pM	16–20	14

FDI – World Dental Federation notation
M – Male
pM – Probably male
NA – Indeterminate gender

We tried to confirm these facts also indirectly by the palaeopathological findings in bones of the postcranial skeleton. All pathological lesions were examined in isolated bones because, as mentioned above, all remains were mixed up in the crypt.

In the sample examined, a suspicion of DISH was discovered in 7 thoracic vertebras from the total number of 130 (5.4 %), which belong to a minimum of one, and to a maximum of three separate individuals. In the first case, two incompletely connected thoracic vertebras had monumental bridging osteophytes in the right frontal surface of the body (*Figure 2*). The next case was a thoracic vertebra with a monumental osteophyte in the right frontal side of the corpus bottom. The last finding consisted of four vertebras (9th–12th thoracic vertebras from one individual). The first vertebra is not fused; the last three ones have already formed a block (*Figure 3*). In the last case the diagnosis of DISH is more likely; on the other hand, in the first two cases it is uncertain because of the small number of vertebras.

Hyperostotic changes were also found in the further three pelvic bones (the frequency of this pathological change reached 7.7 %); this could be related to the DISH illness, too.

Table 4
Concentrations of trace elements in the soil from the crypt

Sample	pH	Ca (%)	Sr (mg/kg)	Zn (mg/kg)	Sr/Zn	Zn/Ca	Sr/Ca
K 1	8.66	13.75	74.9	34.9	2.14	2.54	5.44
K 2	8.91	20.03	73.9	28.2	2.62	1.41	3.69

Table 5
Concentration of trace elements in human teeth – descriptive statistics

	N	Median	Mean	SD
Ca	8	30.10	30.00	0.80
Sr	8	70.55	72.78	12.35
Zn	8	226.00	233.88	56.23
Sr/Zn	8	0.32	0.34	0.13
Zn/Ca	8	7.51	7.81	1.91
Sr/Ca	8	2.34	2.43	0.44

SD – standard deviation

Table 6
Results of one sample Student t-test used for testing the possible diagenetic processes

	Soil sample	t-statistics	p-value
Ca	K1	57.78	<0.0001*
	K2	35.46	<0.0001*
Sr	K1	-0.49	0.6413
	K2	-0.26	0.8040
Zn	K1	10.01	<0.0001*
	K2	10.35	<0.0001*

*statistically significant results (p-value <α=0.05)

In the sample of skeletal remains from St. Catherine's Monastery, one case of DISH was already described, namely in the torso of an individual lying on the crypt floor [23]. The skeletal remains belonged to a male, who died at the age of 44–52 years. The marked hyperostotic changes in the last three thoracic vertebrae with a tendency of connection to the block were observed in this individual. Hyperostotic changes were also present at the upper perimeter of pubic bones, within the *tuberositas iliaca*, *tuber ischiadicum*, and *ramus ossis ischii*, and in both trochanters of the femoral bones. The individual also had ossified rib cartilages that remained separated from the sternum. Trace elements in this individual could not be analysed due to the absence of the skull. However, it cannot be excluded that one of the exhumed skulls had belonged to this individual.

The DISH, known as Forestier disease, is characterised by changes in the spine, which are the result of ossification of the anterior longitudinal ligament and other spinal ligaments. Although, they may occur in all areas of the spine, they are usually most prominent in the thoracic region [30]. In the course of time, the ossification leads to ankylosis of variable numbers of vertebras, but the intervertebral disc spaces and the facet joints are normal in the absence of other pathology. One of the features of the spinal manifestations of DISH is that changes are only found on the right-hand side of the thoracic region [31]. The aetiology of DISH is by no means certain, but one of the earliest suggestions was that it was related to obesity and insulin-independent diabetes mellitus. Later studies have, however, failed to confirm the relationship between DISH and diabetes, but have noted a number

Figure 3
The monumental bridging osteophytes in two thoracic vertebras

of other metabolic abnormalities, including variations in lipid metabolism and hyperuricaemia and elevated levels of growth hormones [32]. DISH occurs frequently in human skeletal remains, particularly in those recovered from monastic cemeteries. It is hypothesised that "the monastic way of life" can predispose to DISH, and obesity as well as diabetes mellitus are considered as the potential co-factors for its development [31].

The familial appearance of DISH was considered to be rare, because the illness usually manifests itself in old age and genetic connections are difficult to evaluate retroactively. On the strength of the familial studies, a highly probable autosomal dominant heredity of DISH was found, and it also explains the connection with some metabolic diseases [33, 34].

CONCLUSION

The results of our analysis indicate that the skeletal remains were most likely members of higher society; they could belong to an aristocratic family. The results of the trace element analysis indicate that the examined population enjoyed a diet rich in proteins. Indirectly, it can also confirm the high prevalence of DISH. However, the high prevalence of Forestier disease can indicate the family relationships between the buried individuals. We know that the teeth sample examined is relatively small, but we try to use all possibilities that would help us to identify a family allegiance of the buried skeletal remains.

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AN UNUSUAL STAB INJURY TO N. PERONEUS COMMUNIS IN A PAEDIATRIC PATIENT

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ABSTRACT

A rare case study of unusual peroneal nerve stab injury in childhood is presented. The completely dissected nerve was urgently treated by neurosurgical microsuture. Minimal clinical and EMG changes were observed during six months, followed by substantial clinical and EMG improvement one year after the surgery. Except for the rare traumatic mechanism, the case study declares a very good clinical result observed even one year after the surgery in the child, if nerve microsuture is urgently performed.

INTRODUCTION

Peripheral nerve injuries in childhood are rare. The most common are lesions of upper extremity nerves, usually due to children's falls and sharp dilacerations caused by broken door windows. Injuries to lower extremity nerves are less common and consist predominantly of compressive or stretch injuries; incised or stab wounds are rare and referred mostly as case studies. This article reports on a rare case study of a 14-year-old girl, who suffered a n. peroneus communis traumatic lesion due to stab injury.

CASE STUDY

While on her summer holidays, a 14-year-old girl fell down from a bicycle in a serious accident, carrying big garden scissors in her backpack (Figure 1). The only injury she suffered was a stab wound in the distal lateral part of her left thigh, caused by the big garden scissors which slipped away from the backpack during the fall. This unbelievable injury mechanism caused disturbance of voluntary foot movement and sensory disturbance, which was correctly detected and diagnosed as n. peroneus injury during emergency surgery treatment. The bleeding was treated by adaptive stitches only and the patient was transferred immediately to the Paediatric Trauma Centre in the Brno Faculty Hospital for

Figure 1
Garden scissors

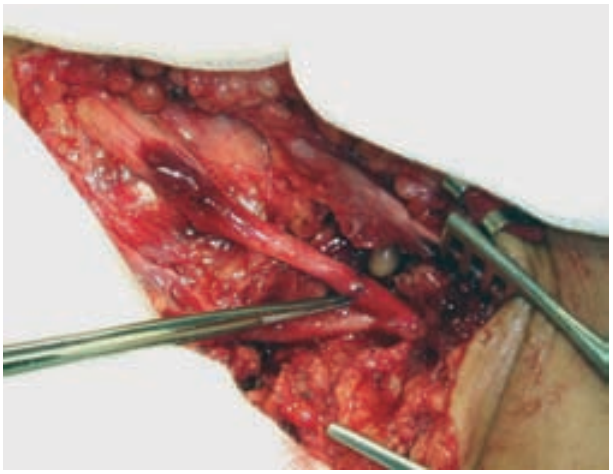

Figure 2
N. peroneus communis after the microsuture

final neurosurgery treatment. Subsequent neurology assessment confirmed a complete left foot-drop with total loss of voluntary ankle and digital dorsiflexion and ankle eversion, tactile hypoesthesia of the lower two-thirds of the lateral leg and dorsum of the foot compatible with a complete left n. peroneus communis traumatic lesion. Based on this examination, immediate neurosurgery revision was performed and microsuture of the completely disrupted common peroneal nerve was carried out (Figure 2).

After the surgery the extremity was fixed in slight flexion by plaster bandage and treatment with antibiotics and neostigmine in a total daily dose of 45 mg was established. The stitches were removed 8 days after the surgery and a limited motion orthosis was applied to enable early physiotherapy, including soft laser therapy applied to the scar. At the time of discharge from the hospital 3 weeks after the surgery the girl walked on crutches only with zero loading on the left leg. Physiotherapy including electric nerve stimulation and neostigmine therapy continued for another 3 months. The patient underwent routine neurosurgery and EMG outpatient examinations at 3 and 6 months after the surgery. Only a minor improvement of sensory loss and a minimal improvement of movement loss were detected clinically, as well as as only an incipient reinnervation finding on EMG (Figure 3). On the contrary, 1 year after the surgery clear reinnervation potentials were recorded on EMG and a substantial functional improvement was detected in clinical examination. The patient was able to walk without any support and without any limitation, slight weakness of the left big toe voluntary dorsiflexion was present together with residual slight tactile hypoesthesia of the left foot dorsum.

DISCUSSION

Traumatic peripheral nerve lesions in childhood are not common. The most frequent injuries are upper extremity nerve lesions [1,2], usually due to children's falls and sharp dilaceration caused by broken door windows. Haase's 5-year study of 43 transsected nerves in paediatric patients included 33 injuries caused by falls through glass doors, windows or by glass fragments [6]. Lower extremity nerve injuries are observed much less often and are mostly caused by compressive or stretch mechanisms; incised or stab wounds are rare in childhood. Eastwood (2005) in an 8-year retrospective study reports on 100 nerve injuries in 94 paediatric patients, of which 81 injuries involved the upper limb, 19 the lower limb [3].

The assessment and treatment of nerve injuries in children are similar to those in adult patients [6]. Preadolescents tend to fracture- or dislocation-related nerve injuries, mostly originating from sports activity. Peripheral nerve injuries in adolescents are similar to those in adults [6]. Common peroneal nerve traumatic injuries are mostly caused by a stretch mechanism due to traffic accidents or job-related injuries in adults. Kim (2004) reports on a group of 318 adult patients operated on for knee–level n. peroneus communis injury; 19 patients underwent end-to-end microsuture repair, and 16 (84 %) of these achieved good recovery within 24 months [5].

The higher nervous system plasticity in childhood and shorter distances needed for axonal regeneration to distal muscles facilitate more favourable clinical and postoperative results in paediatric patients than in adults with the same injuries [6]. The return of functional sensation for children with these injuries was better than for adults with similar lesions (Carmel 1982). Clinical outcome also depends on the causative

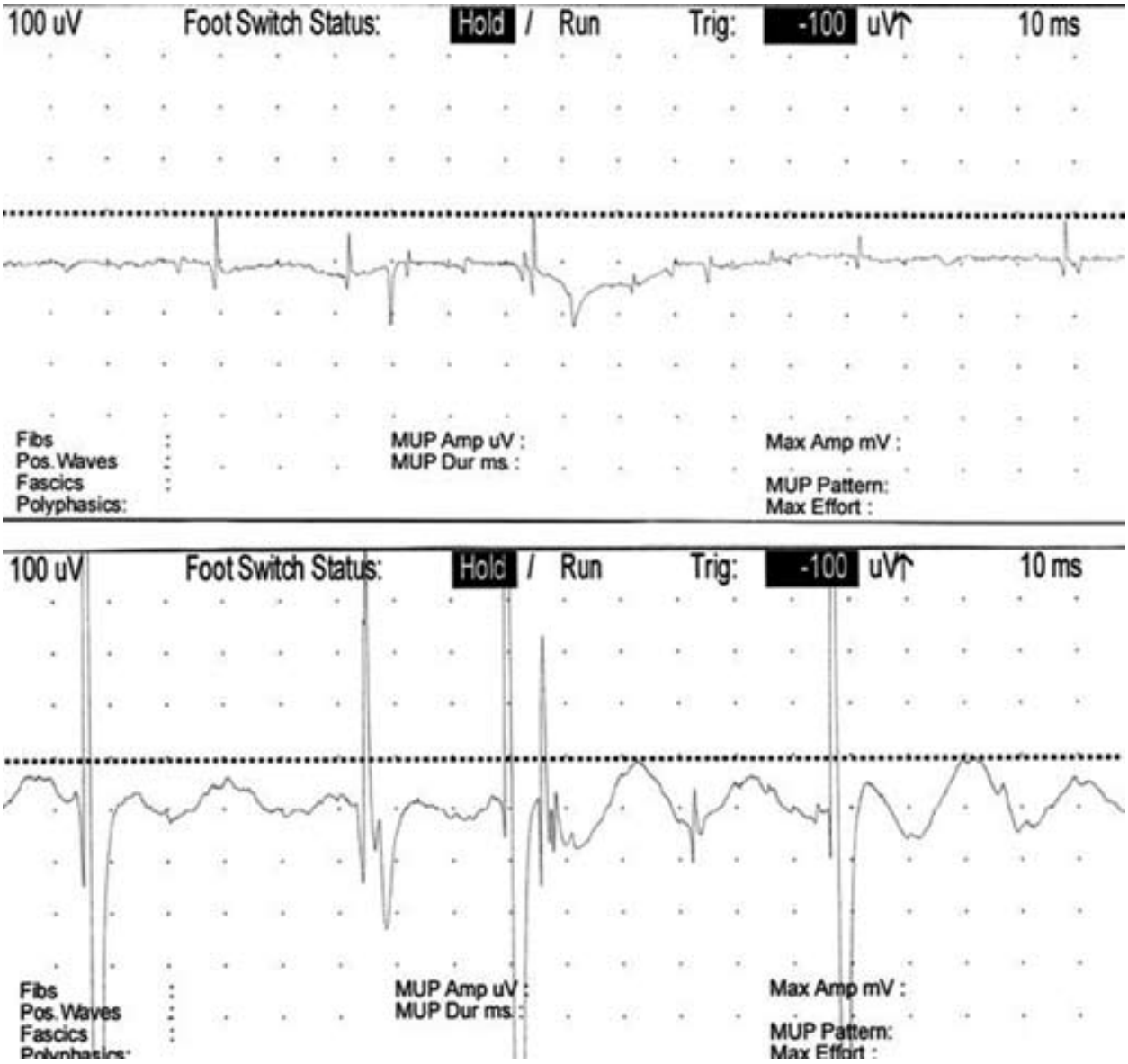

Figure 3
EMG findings

mechanisms of the lesion; sharp injuries and severe knee dislocations had the best recovery [4].

Peripheral nerve injuries in both children and adults require prompt attention to obtain the best outcome. Excellent functional outcome in operated paediatric patients was associated with neurolysis, good outcomes were also associated with nerve grafts and direct repairs. Delayed surgery was associated with fair outcomes [3]. Considering the nerve regeneration following common peroneal nerve repair, it is poorer if compared to other peripheral nerves [4]; the necessity of urgent assessment and specialised neurosurgery treatment is more prominent. Following these requirements, the surgical treatment of common peroneal nerve injuries can be highly rewarding.

CONCLUSION

Urgent peripheral nerve microsuture performed by an experienced neurosurgeon leads to very good postoperative results with almost complete neurological recovery in the child

one year after the surgery. Common peroneal nerve injuries in open wounds should be given specialised neurosurgical treatment at an emergency ward.

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ELECTRODERMAL DIMENSIONAL COMPLEXITY AND SMOKING

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ABSTRACT

Recently, a specific role of nonlinear dynamics and complexity in neural and cognitive processes has been proposed, and there are several reported studies suggesting that smokers might display characteristic changes in the EEG dimensional complexity in comparison to non-smokers. With the aim to extend these findings to autonomic activity, we have examined dimensional complexity in bilateral electrodermal activity (EDA) that reflects limbic modulation influences and may provide information on specific emotional processes related to sympathetic activity. In the present study EDA was assessed in 35 smokers (mean age 23.4, SD=1.4) and 41 non-smokers (mean age 23.2, SD=1.8) during resting conditions. Calculation of dimensional complexity in both groups similarly as in previous reported studies was performed using an algorithm for pointwise correlation dimension (PD2). The results of nonlinear and statistical analysis of EDA records indicate increased complexity during rest conditions (indexed by PD2) in smokers compared to non-smokers (Mann-Whitney test; $p<0.01$), even though EDA measurement does not discriminate the groups (Mann-Whitney test; $p>0.05$). These results present a first supportive evidence that EDA complexity may exhibit an electrophysiological marker that could potentially explain the role of complex dynamics in the autonomic nervous system related to smoking habits and addiction.

INTRODUCTION

According to recent evidence, cognitive brain functions require mechanisms of multiregional functional interaction and integration of multiple and disparate neural activities [1,2]. The extent to which a neural system is dynamically segregated to small subsets of the system that tend to behave independently, or is dynamically integrated with large subsets of the system that behave coherently with synchronous activity, is expressed by neural complexity [3,4]. These findings are of

great significance because an increase in dynamic complexity, linked to an increase in the number of simultaneously active states reflecting the system's degree of freedom, may produce higher neural fragmentation. An increase in complexity is often associated with symmetry breaking and the ability of a system to have different states, which is also associated with a decrease in coherence in space over the long range and with decreased synchronisation [5]. Recently, it has been suggested that applications of the complexity measure to EEG data could be useful for discrimination between different physiological and behavioural conditions [6,7,8]. These findings indicate that the neural complexity may reflect the number of activated oscillating neuronal assemblies in the cortex. These neuronal assemblies can be viewed as basic functional units in brain information processing which constitute associative chains among perceptual, emotional, and cognitive processes creating mental representations [6,7,9,10]. In this context, consistently with psychological findings, Lutzenberger et al. [9] reported that increased dimensional EEG complexity indicates an increase in simultaneously activated neural assemblies, which suggests that the increased complexity is related to enhanced parallel processing and a loosening of associative connections. Further data also show that neural complexity assessed from EEG was substantially higher during imagery than during actual sensory stimulation [8,11,12]. Consistently with these data it has been reported that divergent creative thought is associated with higher EEG complexity than it is in convergent analytical thought [13]. Recent studies also reported that increased complexity of the EEG and EDA has been observed in schizophrenia patients, which likely reflects thought disturbances and attentional deficits [14,15,16]. In this context also other studies indicate that neural EEG complexity reflects the attentional mode of cortical stimulus processing [7,8, 13,17]. The results show that attentional narrowing decreases complexity and causes a reduction in neural competition in connection with an inhibition of neural assemblies irrelevant to task completion during selective attention [18].

Relevance of these findings for the study of smoking behaviour was reported by Pritchard et al. [19], who, in his research of EEG complexity in smokers, proposed that smoking had an "optimising" effect on the neuronal EEG complexity and found that smoking lowered the EEG complexity in subjects whose pre-smoking level was high, did not affect it in subjects whose pre-smoking level was intermediate, and tended to raise it in subjects whose pre-smoking level was low. Similarly, Houlihan et al. [20] suggest that an optimal level of dimensional complexity in smokers should exist for the maximal performance of a given cognitive task. In context with the above findings, several studies indicate that nicotine induces attentional narrowing and can help smokers to filter irrelevant stimuli through increasing attentional selectivity [21,22].

A sensitive measure of attentional functions is also presented by electrodermal activity (EDA) that specifically reflects attention-grabbing stimuli and attention-demanding tasks [23]. EDA is mainly governed by limbic modulation influences and correlates with amygdala activity, but also other structures, such as the ventromedial and dorsolateral prefrontal cortices, anterior cingulate gyrus, parietal lobe, insula, and hippocampus, are involved in EDA modulation [23–25]. EDA typically reflects activity within the sympathetic axis of the autonomic nervous system, which is closely linked to emotional arousal and attentional functions [23].

In the context of previous findings, the aim of the present study is to extend the reported data on EEG complexity in smokers and to test the hypothesis that smokers might display increased EDA complexity in comparison to non-smokers. In order to achieve this goal we have measured resting EDA activity in 31 smokers and 41 non-smokers and performed complexity analysis of the EDA records using an algorithm for pointwise correlation dimension (PD2) similarly as in previous reported studies [16,19,20].

MATERIAL AND METHODS

Participants

The sample was recruited from healthy university students and included 35 smokers (19 males and 16 females; mean age 23.4, SD=1.4; BMI 23.03, SD=2.77) and 41 non-smoking healthy controls (25 males and 16 females; mean age 23.2, SD=1.8 years; BMI 22.9, SD=2.73). The two groups of participants were not statistically significantly different with respect to age and BMI (t-test; $p>0.05$). The exclusion criteria included organic diseases of the CNS, any form of epilepsy, psychiatric disorders, alcohol dependence, and drug abuse according to M.I.N.I. criteria, version 5.0.0 [26].

The evaluation of smoking habits was based on Flay's criteria [27] that define smoking maintenance as a stage of regular use from occasional to daily smoking leading to the development of chronic, daily patterns of nicotine use. For the purpose of this study smokers were allowed to smoke ad libitum according to their habits. The main features of smoking behaviours were described by mean consumption of cigarettes (5.8±3.2 per day) and mean continuation of smoking (4.8±1.6 years). Nicotine dependence was assessed using Cigarette Dependence Scale (CDS) with a mean score of 26.9±10.5 [28] (Cronbach's alpha 0.84, test-retest reliability after week 0.83).

All the participants were right-handed according to Waterloo Handedness Questionnaire [29]. The questionnaires were administered before EDA measurement in a quiet room with the help of a psychologist. The study was approved by the

University ethical committee and all the participants gave their written informed consent.

EDA measurement

EDA was recorded bilaterally using a two-channel SAM unit and Psylab software (Contact Precision Instruments) connected to a personal computer. The measurements were performed in a quiet room at a room temperature of about 23 °C. During the experiment the participants sat in a comfortable chair. The measurement was performed using two pairs of Ag/AgCl electrodes (8 mm diameter of active area) filled with electroconductive paste, which were attached to the medial phalanges of the index and the middle finger of each hand. EDA measurement was performed at a sampling frequency of 1000 Hz. After five minutes of resting state with the eyes closed, the experimental EDA recording with closed eyes during rest and lasting two minutes was performed.

Data analysis

A practical approach to studying complex dynamical systems is the method of time-series analysis. A postulate of this method is that every dynamic system, for example the human brain and its functions, is governed and determined by a number of independent variables. Nevertheless, any real measurement performed on the system cannot provide information about all the variables because a system of high complexity such as the human brain is multidimensional. Time-series analysis therefore represents a mathematical approximation that enables the reconstruction of certain variables underlying the multidimensional dynamics from data obtained from the system during the time [30]. The data may provide, for example, a psychophysiological measurement performed on the system during an experiment. Since observational data reflect only a few real independent variables of a system, approximation of the dynamic system behaviour uses a finite number of (mathematically reconstructed) variables to approximate the states of the system. The multidimensionality of the dynamic system is therefore approximated by an embedding dimension that represents the dimensionality contained (or embedded) in the data. In the analysis, the 120-second EDA records in resting conditions were processed using the Dataplore software package, considered as one of the most well-known software implementations for time-series analysis. Skinner's algorithm for a pointwise correlation dimension (PD2) was used in the analysis [31]. The PD2 algorithm for this calculation was selected because it has been shown useful in detecting the complexity of phase transitions for non-stationary time series of dynamic systems [30]. Calculation of the PD2 is based on the formula

$PD2(i) = logC(r,i)/log(r)$,

where C(r,i) is a pointwise correlation integral which gives a sort of time average over the whole signal providing a value for each time point of the signal and *r* is the distance reflecting the radius of the neighbourhood around a certain point (between 0 and 1).

Statistical differences for PD2 values between the groups of smokers and non-smokers were assessed in a statistical evaluation which included the calculation of means, standard deviations (SD), and their comparison by a non-parametric Mann-Whitney U test for independent samples using the Statistica software package, version 8.0.

RESULTS

The results of nonlinear data analysis show that EDA complexity described using PD2 values in rest conditions is significantly higher in smokers in comparison to non-smokers on both sides (Mann-Whitney test; $p<0.05$) (Table 1). Conversely, statistical analysis did not reveal any significant difference in the values of electrodermal activity during resting conditions between smokers and non-smokers (Mann-Whitney test; $p>0.05$) (Table 1). A correlation analysis did not show any significant relationship between EDA and CDS (Spearman r left 0.06; right 0.03; $p>0.05$) or PD2 and CDS score (Spearman r left 0.17; right 0.27; $p>0.05$).

Table 1

Values of EDA (in microsiemens) and EDA complexity (PD2) in smokers and non-smokers during rest conditions.

	Smokers	Non-smokers	Mann-Whitney test		
PD2, EDA	(N=35)	(N=41)			
	Mean ± *SD*	*Mean* ± *SD*	*U*	*Z*	*p*
Left PD2	0.845 ± 0.106	0.719 ± 0.106	441.5	2.069435	0.04
Right PD2	0.856 ± 0.110	0.741 ± 0.110	394.5	2.614328	0.01
Left EDA	2.093 ± 1.083	2.308 ± 1.727	533.5	0.158904	0.87
Right EDA	2.157 ± 0.920	2.288 ± 1.720	484.5	0.781808	0.43

DISCUSSION

The results of the present study are in agreement with the proposed hypothesis that smokers in comparison to non-smokers display higher neural complexity in the resting state. These findings suggest that the dynamic EDA complexity indicating nonlinearities in limbic modulation influences may reflect changes in neural dynamics and cognitive functions that distinguish smokers and non-smoking healthy controls. In the context of the above findings higher dynamic complexity reflects an increased level of synchronisation between items of the functional network that can be interpreted as an increased occurrence of dynamically segregated subsets in the neural system [10]. In this context, Lutzenberger et al. [18] presented results showing that attentional narrowing decreases complexity and causes a reduction in neural competition in connection with an inhibition of neural assemblies irrelevant to task completion during selective attention.

Because of typical attentional deficits in smokers reported in previous studies, the finding of increased neural complexity in smokers in the present study suggests that the deficit in attentional narrowing might be related to information overload caused by defective attentional filtering and frontal lobe executive dysfunction in smokers [21,32,33,34]. These data are in agreement with findings of impaired selective attention, self-regulation, and behavioural regulation associated with smoking [35,36,37].

For example, Spinella [34] shows positive correlations between smoking and impairment on putative measures of orbitofrontal dysfunction and suggests the hypothesis that an orbitofrontal dysfunction related to selective attentional deficit predisposes an individual to nicotine and tobacco abuse. Similarly, Powell et al. [33] reported an effect of nicotine on enhancing the ability to inhibit reflexive eye movements controlled from brain structures participating in executive functions. There is also growing evidence that chronic use of addictive substances (alcohol, nicotine, psychomotor stimulants, and opiates) is associated with impairments of impulse control (response inhibition) and behaviour that has been linked with the activity in the orbitofrontal cortex [32,33,34]. Conversely, nicotine induces attentional narrowing and can help the smoker to filter irrelevant stimuli by increasing attentional selectivity [21,22].

Recent findings also indicate that smokers present a subpopulation with a significantly increased level of stress [38–41]. This specific condition could be reflected in the level of neural complexity because preliminary data suggest that a heightened level of ACTH impairs the attentional filtering and leads to an increase of the EEG dimensional complexity [8]. Consequently, attention in persons with a heightened level of ACTH and EEG dimensional complexity is distracted more easily by irrelevant stimuli [8].

These results are in agreement with preliminary data by Pritchard et al. [19] suggesting that smoking lowered the EEG complexity in subjects whose pre-smoking level was high, did not affect it in subjects whose pre-smoking level was intermediate, and tended to raise it in subjects whose pre-smoking level was low. Houlihan et al. [20] also suggest that an optimal level of dimensional complexity should exist for the maximal performance of a given cognitive task and may be influenced by smoking.

In this connection it is likely that normal information processing could require intermediate levels of dynamical complexity [10] and smoking could help smokers tune in their brain for optimal functioning. Although it is still largely unanswered which specific acute interoceptive and behavioural effects of nicotine are the most reinforcing [39,42], the self-medication hypothesis proposed by Khantzian [43] is a useful way to explain the higher rates of smoking among subjects who need to regulate their cognitive processes, moods, or coping with stress.

In summary, the findings of the present study suggest that specific attentional deficits related to attentional narrowing could be reflected by EDA dimensional complexity, thus enabling to distinguish smokers from non-smoking healthy controls. These results present a first supportive evidence that EDA complexity may present an electrophysiological marker that could potentially explain the role of complex dynamics in the autonomic nervous system related to smoking habits and addiction.

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HYPOTHYROIDISM IN SENIORS HOSPITALISED IN INPATIENT GERONTOPSYCHIATRIC WARDS IN MENTAL HOSPITAL KROMĚŘÍŽ

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ABSTRACT

The aim of our study was to observe prevalence of undiagnosed latent or clinically apparent hypothyroidism in senior patients hospitalised in gerontopsychiatric wards of Mental Hospital Kroměříž. Correlation of findings with the diagnosis of depression and dementia in these patients. Comprehensive psychiatric assessment, diagnostic criteria ICD–10 for depression and dementia, Geriatric Depression Scale (GDS), Mini-Mental State Examination (MMSE), blood levels of thyroid stimulating hormone (TSH), and the free fraction of thyroxine (fT4) were checked in laboratory, both in the basic and the control group of patients. High prevalence of thyroidism was observed in the group of seniors hospitalised in the inpatient gerontopsychiatric ward of Mental Hospital Kroměříž and association between scores on the Geriatric Depression Scale (GDS), scores in Mini-Mental State Examination (MMSE), and hypothyroidism was found. We assume that early detection and early treatment of hypothyroidism can be useful in the management of dementia and depression in gerontopsychiatric patients, and as for younger age, both early detection and early treatment of hypothyroidism can be helpful in the prevention of dementia and depression in older age.

INTRODUCTION

Hypothyroidism is one of the most common endocrinological conditions in population. It occurs with 10% prevalence in general population, dominantly in women and older people [1, 2, 3, 4]. The diagnosis of hypothyroidism is established either in case of clinical symptoms of hypothyroidism (non-specific symptoms like fatigue, lethargy, depression, general malaise, constipation, weight gain, hoarse voice, dry skin or dry hair, oedema of hands, limbs or eyelids [1], or laboratory signs: increase of thyroid-stimulating hormone (TSH)

or decrease of the free fraction of thyroxine (fT4). Hypothyroidism remains very often undiagnosed or underdiagnosed, especially in case of clinically inapparent hypothyroidism, although various symptoms such as fatigue, weight gain or obesity, dyslipidemia or other symptoms of the metabolite syndrome, changes in arterial blood pressure, and affective disorders can appear moderately [1, 2]. Hypothyroidism is sometimes observed in psychiatric conditions such as depression, mania or schizophrenia [2, 4]. If hypothyroidism interferes with depression, remains undiagnosed and untreated, then the therapy of depression is very difficult and sometimes impossible.

Research of subclinical hypothyroidism in gerontopsychiatric patients was done only rarely [3] and its results are not consistent.

METHODS

Basic group: All senior patients (65+) admitted to the inpatient gerontopsychiatric ward in Mental Hospital Kroměříž underwent a comprehensive psychiatric assessment, followed by laboratory checks. The diagnosis of depression and dementia was established according to the diagnostic criteria of ICD–10 classification. The Geriatric Depression Scale (GDS) and Mini-mental state Examination (MMSE) were used for the evaluation of the severity of depression and cognitive impairment. Blood levels of the thyroid-stimulating hormone (TSH) and the free fraction of thyroxine (fT4) were checked in laboratory.

Control group: Young patients (–30) admitted to the inpatient psychotic ward in Mental Hospital Kroměříž with psychotic disorders, not fulfilling diagnostic criteria either for depression or dementia: the Geriatric Depression Scale (GDS) and Mini-mental State Examination scores (MMSE) were used as control scores to the basic group; blood levels of the thyroid-stimulating hormone (TSH) and the free fraction of thyroxine (fT4) were checked in laboratory.

In both groups: The cut-off levels for hypothyroidism were: 4.0 mIU/L for TSH and 8 pmol/l for fT4. Verbal informed consent was given by all patients for participation in our study.

RESULTS

Basic Group (65+)

	Men	Women	Both sexes
Patients	53	78	131
Age (mean)	67.2	69.8	68.7
SD of age	5.6	9.4	7.3
MMSE score (mean)	18.9	16.8	17.5
SD of MMSE score	6.7	9.2	7.9
GDS score (mean)	7.4	5.5	6.2
SD of GDS score	3.4	3.2	3.3
TSH level (mean)	3.9	4.6	4.1
SD of TSH level	1.2	1.4	1.3
fT4 level (mean)	13.2	10.1	12.4
SD of fT4 level	4.2	4.6	4.5

SD – standard deviation, MMSE – Mini-mental State Examination, GDS – Geriatric Depression Scale, TSH – thyroid-stimulating hormone, fT4 – free fraction of thyroxine

	Men	Women	Both sexes
Latent hypothyroidism (patients)	8	22	30
in %:	15.1	28.2	22.9
Clinically significant hypothyroidism (patients)	1	10	11
in %:	1.9	12.8	8.4

Control group (–30)

	Men	Women	Both sexes
Patients	50	55	105
Age (mean)	28.8	27.6	28.1
SD of age	4.2	4.1	4.1
MMSE score (mean)	28.3	28.5	28.4
SD of MMSE score	2.6	2.8	2.7
GDS score (mean)	4.2	4.8	4.6
SD of GDS score	1.9	2.1	2.0
TSH level (mean)	3.8	4.1	3.9
SD of TSH level	0.9	1.1	1.0
fT4 level (mean)	17.4	16.8	17.1
SD of fT4 level	3.9	4.1	4.0

SD – standard deviation, MMSE – Mini-mental State Examination, GDS – Geriatric Depression Scale, TSH – thyroid-stimulating hormone, fT4 – free fraction of thyroxine

	Men	Women	Both sexes
Latent hypothyroidism (patients)	3	9	12
in %:	6.0	16.4	11.4
Clinically significant hypothyroidism (patients)	1	5	6
in %:	2.0	9.1	5.7

RESULTS OF CORRELATION ANALYSIS

– a higher prevalence of clinically inapparent hypothyroidism in the basic group (65+) compared to the control group (–30) (p=0.04);

- the difference in the prevalence of clinically apparent hypothyroidism between both groups was statistically insignificant (p=0.09);
- a higher prevalence of thyroidism in women compared to men in basic groups (p=0.007) and in the control group (p=0.03);
- a significant correlation was found between the Geriatric Depression Score level (GDS) and the plasma level of the thyroid-stimulating hormone (TSH) in both sexes in the basic group (men: p=0.03; women: p=0.02);
- no correlation between the Geriatric Depression Score level (GDS) and the plasma level of the free fraction of thyroxine (fT4) was found in the basic group (men: p=0.12; women: p=0.10);
- a significant correlation between Mini-mental State Examination scores (MMSE) and the plasma level of the thyroid-stimulating hormone (TSH) in both sexes in the basic group (men: p=0.025; women: p=0.022);
- no correlation between Mini-mental State Examination scores (MMSE) and the plasma level of the free fraction of thyroxine (fT4) was found in the basic group (men: p=0.09; women: p=0.11).

DISCUSSION

We observed a significant correlation between the Geriatric Depression Score level (GDS) and the plasma level of the thyroid-stimulating hormone (TSH) in both sexes in groups of senior patients (65+) hospitalised in the inpatient gerontopsychiatric ward and a significant correlation between Mini-mental State Examination scores (MMSE) and the plasma level of the thyroid-stimulating hormone (TSH) in both sexes in groups of senior patients (65+) hospitalised in the inpatient gerontopsychiatric ward. Compared to Park's study [5], we observed these correlations both for clinically apparent and clinically inapparent hypothyroidism. We assume that early detection of hypothyroidism (clinically apparent or inapparent) and its early treatment can improve depression and perhaps dementia in gerontopsychiatric patients. Findings of different research done in the world are sometimes consistent and sometimes inconsistent with our assumption (6, 7, 8, 9). Further research of hypothyroidism in gerontopsychiatric patients, with a view to lifelong prevention of depression and dementia in general population, is recommended.

CONCLUSION

Hypothyroidism (both clinically inapparent and clinically apparent) is not a rare condition in seniors (+65) hospitalised in the inpatient gerontopsychiatric wards of Mental Hospital Kroměříž; its prevalence is high compared to the group of young patients (–30) hospitalised in the inpatient psychotic wards of the same institution.

We found a statistically significant correlation between Mini-mental State Examination scores (MMSE) and the plasma level of the thyroid-stimulating hormone (TSH) in seniors of both sexes (+65) hospitalised in the inpatient gerontopsychiatric wards of Mental Hospital Kroměříž. Moreover, a statistically significant correlation between the Geriatric Depression Score level (GDS) and the plasma level of the thyroid-stimulating hormone (TSH) was found in seniors of both sexes (+65) hospitalised in the inpatient gerontopsychiatric wards of Mental Hospital Kroměříž.

We assume that early detection and early treatment of hypothyroidism can be useful in the management of dementia and depression in gerontopsychiatric patients, and as for younger age, both the early detection and early treatment of hypothyroidism can be helpful in the prevention of dementia and depression in older age.

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CROHN'S DISEASE ACTIVITY VERSUS EXTENT OF DNA DAMAGE/ REPAIR AND VARIABILITY IN THE RAGE GENE

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LIST OF ABBREVIATIONS

AGEs – advanced glycation end-products
CD – Crohn's disease
CDAI – Crohn's Disease Activity Index
CML – carboxymethyllysine
DAMPs – damage-associated molecular pattern proteins
DNArc – DNA repair capacity
DNAssb – DNA single strand breaks
EN-RAGE – extracellular newly identified ligand of receptor for advanced glycation end-products
Ery-GSH – erythrocyte glutathione
Ery-SOD – erythrocyte superoxide dismutase
GWAS – genome-wide association study
IBD – inflammatory bowel disease
NFkB – nuclear factor kB
NOD – nucleotide-binding oligomerisation domain protein
PAMPs – pathogen-associated molecular patterns
PCDAI – Pediatric Crohn's Disease Activity Index
PCR – polymerase chain reaction
P-MDA – plasma malondialdehyde
PRR – pattern-recognition receptor
P-tAOC – plasma total antioxidant capacity
RAGE – receptor for advanced glycation end-products
ROS – reactive oxygen species
SNP – single nucleotide polymorphism
S-CRP – C-reactive protein
sRAGE – soluble receptor for advanced glycation end-products,
TBARS – thiobarbituric acid
TNFa – tumour necrosis factor a
WB-GPx – whole blood glutathione peroxidase.

ABSTRACT

The aim of the study was to investigate the relationships between the extent of oxidative stress and DNA damage/repair, disease severity, and selected genetic variants in the RAGE gene in Crohn's disease (CD) patients. The study comprised a total of 46 subjects with CD and 99 control subjects. Disease activity was characterised by the Crohn's Disease Activity Index (CDAI) and paediatric CDAI (PCDAI) and complications requiring surgery (abscess, fistula or stenosis). Selected markers of oxidative stress (superoxide dismutase (Ery-SOD), glutathione peroxidase (WB-GPx), total plasma antioxidant capacity (P-tAOC), reduced glutathione (Ery-GSH) and malondialdehyde (P-MDA)), DNA damage (DNA single-strand breaks), and repair capacity detected (DNArc) by Comet assay were ascertained. Four SNPs in the RAGE gene were detected by PCR. A significant correlation between CD duration and DNArc was identified in adult patients ($r = 0.44$, $P = 0.039$), and adult CD subjects had significantly higher Ery-SOD levels ($P = 0.0005$). In children, both CDAI and PCDAI significantly correlated with P-tAOC ($r = –0.5$, $P = 0.02$ and $r = –0.53$, $P = 0.013$, respectively) and also with WB-GPx ($r = –0.5$, $P = 0.03$ and $r = –0.51$, $P = 0.018$, respectively). Child CD patients also exhibited a significant correlation between age and Ery-GSH and P-MDA ($r = –0.6$, $P = 0.005$ and $r = 0.5$, $P = 0.02$, respectively). Significant differences in Ery-GSH and P-MDA were found between CD patients with and without history of complications ($P = 0.05$ and $P = 0.013$, respectively). There was no statistically significant relationship between the carrier state of any of the four RAGE SNPs and oxidative stress, DNA damage or DNA repair parameters analysed. In conclusion, intestinal inflammation is reflected in selected circulating markers of oxidative and genotoxic stress.

INTRODUCTION

Crohn's disease (CD) represents a form of inflammatory bowel disease (IBD), a category comprising several other distinct intestinal pathologies such as ulcerative colitis, indeterminate colitis, and other rarer forms. CD is generally believed to result from an inappropriate immune response to the bacterial flora in the intestine that occurs in genetically susceptible individuals with simultaneous environmental contribution [1]. Although specific pathogens (e.g. *M. paratuberculosis*, *L. monocytogenes, E. coli* or measles virus) cannot be excluded, CD results more likely from the breakdown of physiological hyporesponsiveness to commensal resident microflora (more than 500 different bacterial species) and dietary antigens [2]. Histopathologically, CD is characterised by the formation of transmural granulomatous inflammatory lesions in any part of the gastrointestinal tract from the mouth to the anus, though most commonly in the terminal ileum and proximal colon, with possible extraintestinal manifestations (such as arthritis, osteoporosis, skin lesions, hepatopathy or uveitis). CD exhibits several typical features such as manifestation in young adults (2nd–3rd decades), ethnic differences in prevalence, familial aggregation, and approx. a 45% concordance rate in monozygotic twins (indicating the role of genetic factors) and growing incidence in Europe and North America (indicating the involvement of environmental factors, e.g. smoking, diet, increased sanitation, etc.). The natural course of CD involves recurrent exacerbations (manifested by abdominal pain, diarrhoea, cramping, fever, or enterorrhagia) with remissions. CD is accompanied by frequent development of gastrointestinal tract complications such as fistulae, stenoses or gut obstructions as well as systemic complications such as anaemia, malabsorption, weight loss or failure to thrive in children, etc.

Our understanding of the CD aetiopathogenesis has recently moved greatly forward thanks to the results of genome-wide association (GWAS) and linkage genetic studies [3], experimental animal models, and advanced immunological techniques. Recent evidence suggests that deficient innate immune response (genetically predisposed) plays an important role in the initiation of inflammation characterised by concomitant pathological adaptive immune T-cell response leading to the intestinal barrier defect and tissue destruction [4]. In healthy individuals the intestinal mucosa is in a state of tolerance or, rather, "controlled" inflammation against intestinal microflora due to a balanced activation of innate immune response by the intestinal immune system [1]. This control relies largely on the ability of the host to recognise conserved microbial motifs (pathogen-associated molecular patterns, PAMPs) comprising diverse molecules such as lipopolysaccharide, peptidoglycan, flagellin, etc. by the pattern-recognition receptors (PRRs). The two important members of PRRs synergistically involved in intestinal immunity are the transmembrane Toll-like receptor family and their intracellular counterparts NLR (nucleotide-binding oligomerisation domain, NOD-like) receptor family expressed by mononuclear, dendritic, epithelial, and Paneth cells [4]. NOD2 detects muramyl dipeptide, the largest PAMP of both Gram-negative and Gram-positive bacteria. The NOD downstream signalling pathway includes NFkB and caspase 1 inflammasome activation, induction of proinflammatory cytokines (such as TNFα and IL–1β), and stimulation of antimicrobial α-defensins by Paneth cells [5]. Among multiple loci shown to predispose to the CD CARD15 gene located on chromosome 16 encoding NOD2 repeatedly exhibited the strongest association with CD, predominantly in subjects with small bowel disease [6–8]. The carriers of several distinct variants in this locus (responsible for abnormal recognition

of intestinal bacterial products resulting in deficient regulation of α-defensin production by Paneth cells) have a 2 to 4 times higher relative risk of CD in the heterozygote state and a 20 to 40 times higher one in the homozygote state [9].

Although the exact functional impact of CD-associated single nucleotide polymorphisms (SNPs) remains unclear so far, it has been recently proposed that defective expression of α-defensins might be a bridge to the abnormal adaptive immune response in CD, namely maturation of dendritic cells and polarisation of Th1/Th2 response [10, 11]. Unrestrained bacterial invasion and antigen uptake by intestinal M-cells with subsequent activation of mucosal antigen-presenting cells (dendritic cells and macrophages) and their maturation in mesenteric lymph nodes stimulate them to produce IL–12, which induces a Th1 cytokine response (IL–1, IL–6, TNFα and INFγ) and, at the same time, suppresses Th1/Th3 response (IL–10 and TGFβ) [1]. This facilitates recruitment and infiltration by auxiliary effector cells (mainly neutrophils) and amplification of inflammation. Another important contributor to the neutrophil recruitment (and thus proinflammatory phenotype) is activation of the IL–23/Th17 pathway, which is documented by another strong and replicated genetic association (GWAS) of CD with polymorphisms in the IL–23 receptor gene [12]. Chronic nature of CD (unlike acute enteral infection) is, however, attributed to the persistent mucosal regulatory CD4+/CD25+ T-cell (T_{REG}) defect (due to a lack of IL–10/TGFβ stimulation) and defective apoptosis of activated T cells (resistance is due to their sustained stimulation by IL–6) [1, 13]. Excessive and persistent Th1 effector cell response in the gut mucosa is marked by massive TNFα production. Unlike activated T cells resistant to apoptosis, cytokines (TNFα and INFγ) increase the permeability of intestinal barrier acting on the epithelial tight junctions and induce apoptosis of the intestinal epithelium (characterised by low antioxidant capacity).

Influx of neutrophils into the intestinal mucosa is a hallmark of an active CD and the very executor of tissue destruction, intestinal barrier defect, and perpetuation of stimulation by bacterial antigens [14]. Neutrophils mediate tissue damage both directly – via release of matrix metalloproteinases – and indirectly via production of reactive oxygen species (ROS) by NADPH oxidase and subsequent oxidative modification of macromolecules and by release of S100 proteins (calgranulins). The latter category represents a family of more than 20 proteins produced by monocytes and polymorphonuclears with important intracellular functions (cell cycle control, calcium homoeostasis, migration, cytoskeleton organisation, etc.) which, upon release to the extracellular space, acquire multiple novel functions (such as antimicrobial activity, chemotaxis, induction of apoptosis, ROS scavenging, etc.) and, at the same time, serve as markers of inflammation called damage-associated molecular pattern proteins (DAMPs) [15]. Calprotectin (complex S100A8/S100A9) serves as a potent chemotactic factor and induces endothelial adhesion and extravasation of phagocytes to the site of inflammation. S100A12, another calgranulin previously known as EN-RAGE (extracellular newly identified ligand of receptor for advanced glycation end-products) has been implicated in a novel inflammatory axis involving the RAGE/NFkB pathway. Measurement of faecal calprotectin proved to be a promising non-invasive and fairly specific marker of intestinal inflammation, especially in children [16, 17]. Similarly, S100A12 was shown to be specifically and sensitively associated with IBD in children [18].

Release of ROS and S100A proteins by neutrophils in the intestinal wall of IBD patients not only represents parallel pathogenic events but also mutually aggravating processes, since S100A proteins can be posttranslationally modified, e.g. by carboxymethyllysine (CML), an advanced glycation end-product (AGE) whose production is enhanced by the myeloperoxidase system of phagocytes at sites of inflammation [19]. Presence of CML-modified S100A proteins has been detected in inflamed regions of gut bioptic specimens from CD patients together with up-regulated expression of RAGE and NFkBp65 [20]. Both AGEs and S100A12 are ligands of RAGE which, upon engagement, triggers or at least significantly contributes to the long-lasting activation of NFkB, since CML-induced NFkB activation was reduced in the presence of synthetic soluble RAGE (sRAGE) [20]. Interestingly, a poor expression of endogenous sRAGE, a splice variant of the full-length form with supposedly protective (neutralising) activity, has been shown in the intestinal mucosa compared to the serum levels in both non-IBD and IBD subjects [21]. This suggests that sustained topical elevation of RAGE ligands can trigger unrestricted inflammatory response in tissues with genuinely limited antioxidant capacity such as the intestinal epithelium. An overview of suggested pathogenic mechanisms relating to the S100A/AGE – RAGE signalling axis is shown in Figure 1.

Such a chronic inflammation inevitably contributes to the increased oxidative damage of the macromolecules which, in case of DNA, requires an energetically costly repair. Currently, there is limited knowledge regarding contribution of the oxidative modification of macromolecules and their subsequent repair to the CD pathogenesis. Genetic and subsequent functional variability in the pro- and antioxidant system, DNA repair system as well as other proinflammatory mediators (e.g. RAGE) can very well establish a secondary factor individually modulating the CD course and its severity. Based on this hypothesis, the aim of the current study was to investigate the relationships between the extent of oxidative stress and DNA damage/repair, disease severity, and selected common genetic variants in the RAGE gene in CD patients. Providing

Table 1
Patient's clinical characteristics

	All subjects (n = 46)	Adults (n = 25)	Children (n = 21)
Gender	22 males / 24 females	14 males / 11 females	8 males / 13 females
Median age (yrs)	20.5 [15–31]	30 [24–37]	15 [13–17]
CD duration (yrs)	3 [2–8]	4 [2–11]	3 [2 –5]
Median age at CD onset (yrs)	14.5 [4–51]	24 [12–51]	12 [4–15]
CD activity – CDAI	53 [10–295]	48 [10–154]	57 [20–295]
CD activity – PCDAI	- -	- -	20 [5–67.5]
CD complications	34 (73.9 %)	19 (76 %)	15 (71.4 %)

Data expressed as a median [interquartile range].

Table 2
Parameters of oxidative stress, DNA damage, and DNA repair

Parameter	CD adults (n = 25)	Control adults (n = 88)	P	CD children (n = 21)	Control children (n = 11)	P
Ery-SOD (U/g Hb)	1397.0 [1292.0–1496.0]	1233.0 [1172.0–1309.0]	0.0005	1467.0 [1237.0–1495.0]	1452.0 [1359.0–1532.0]	NS
WB-GPx (U/g Hb)	55.5 [50.5–67.2]	59.3 [54.3–67.2]	NS	58.4 [45.1–67.9]	47.3 [44.4–52.7]	NS
P-tAOC (mmol/L)	1.49 [1.31–1.67]	1.48 [1.41–1.54]	NS	1.47 [1.3–1.56]	1.44 [1.33–1.49]	NS
Ery-GSH (mmol/L)	2.18 [2.04–2.36]	2.02 [1.8–2.3]	NS	2.14 [2.02–2.25]	2.21 [2.12–2.37]	NS
P-MDA (umol/l)	1.75 [1.4–2.13]	2.08 [1.8–2.3]	NS	1.76 [1.47–2.13]	1.83 [1.55–2.23]	NS
DNAssb (n/10^9 Da DNA)	0.35 [0.21–0.71]	---	NS	0.48 [0.15–0.63]	0.36 [0.28–0.43]	NS
DNArc (n/10^9 Da DNA)	1.58 [0.95–2.3]	---	NS	2.14 [2.0–2.3]	1.44 [1.38–1.54]	NS
DNARI [= DNArc/DNAssb]	3.4 [1.98–9.28]	---	NS	4.3 [3.8–8.45]	4.05 [3.16–4.49]	NS

Data expressed as a median [interquartile range], comparisons performed using Mann-Whitney test.

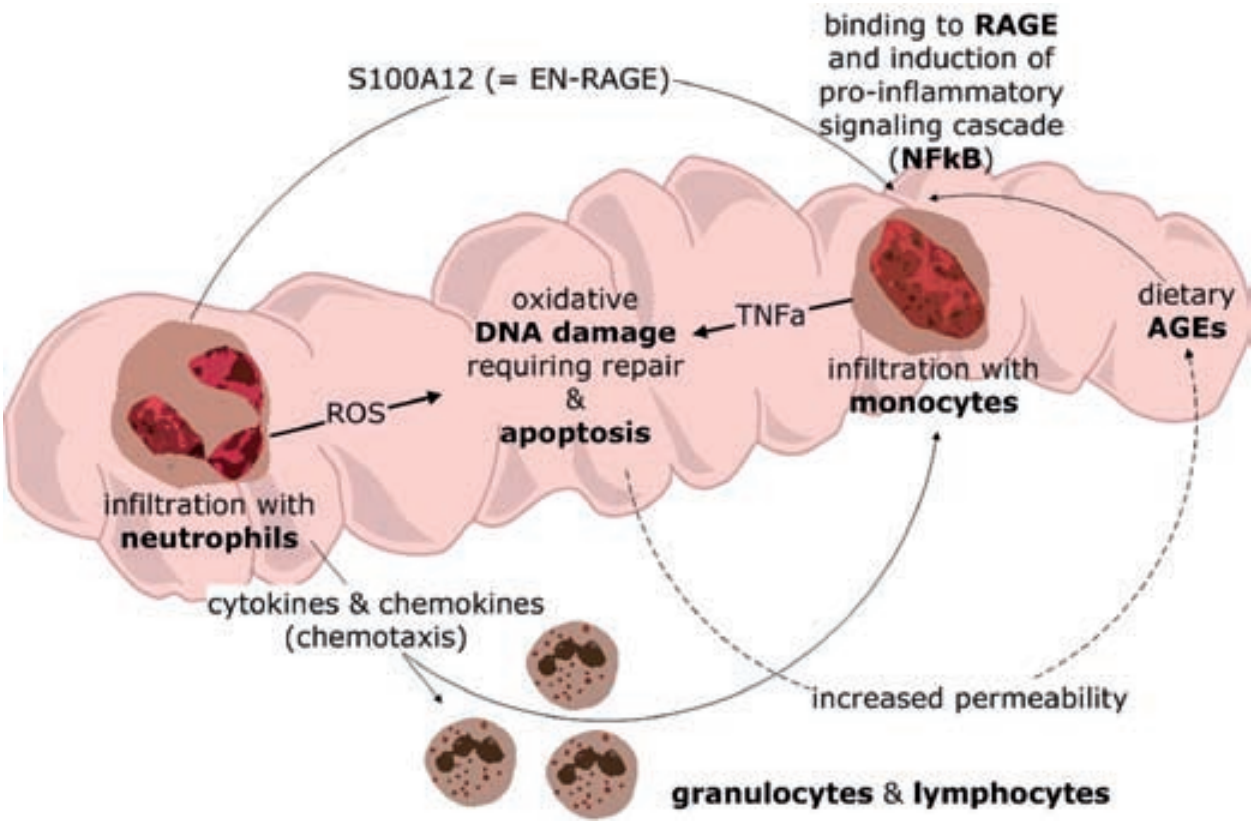

Figure 1
Release of ROS and S100A12 proteins in intestinal epithelium with up-regulated expression of RAGE. Increased release of reactive oxygen species with subsequent demand for a repair of oxidative damage together with AGE/S100A12 induced RAGE – NFkB activation perpetuate inflammatory process and impairment of intestinal barrier. This can allow exogenous RAGE ligands (dietary Maillard reaction products or AGEs) to act as an aggravating mechanism in IBD patients.

that the data suggest the pathogenic role of the proposed processes incl. RAGE signalling cascade, it might be worth studying the impact of enrichment of the diet with exogenous RAGE ligands (Maillard reaction products or AGEs) as a potentially aggravating factor of CD progression.

SUBJECTS AND METHODS

Subjects and CD diagnosis

The case-control study comprised a total of 46 subjects (22 men, 24 women) with established CD (cases). Adult CD subjects (n = 25) were followed up in the Gastroenterology Unit of the Department of Internal Medicine, Charles University Hospital, Hradec Králové, Czech Rep.; children (n = 21) were followed up in the Gastroenterology Unit of the Dept. of Paediatrics, Charles University Hospital, Plzeň, Czech Rep. Clinical characteristics of the subjects are given in Table 1. Diagnosis of CD was based on clinical symptoms, diagnostic imaging (ultrasound, colonoscopy or upper endoscopy, computer tomography), and histopathology verification from bioptic material. All subjects were treated by salicylates, subsets by corticosteroids and/or other immunosuppressive drugs and/or biologicals (anti-TNFα antibody). CD activity was assessed by the Crohn's Disease Activity Index (CDAI), whose calculation is based on the frequency of stools, rating of abdominal pain, presence of intestinal and extraintestinal complications, haematocrit, and body weight. CDAI < 150 was considered as an inactive disease, CDAI > 150 as an active one. For children, paediatric CDAI (PCDAI) was calculated based on (i) subjective reporting of the degree of abdominal pain, stool pattern, and general well-being, (ii) presence of extraintestinal manifestations (fever, arthritis, rash, and uveitis), (iii) physical examination findings (weight and height), (iv) haematological and biochemical parameters (haematocrit, erythrocyte sedimentation rate and serum albumin). CD complications were defined as an event requiring surgery (i.e. abscess, fistula or

stenosis). Controls (n = 99) were healthy unrelated subjects negative for Crohn's disease or any gastrointestinal symptomatology recruited from the clientele of several general practitioners [88 adults (42 men and 46 women) with a median age of 31.0 [IQR 27.0–41.5] years] and paediatric practitioners [11 children (6 men and 5 women) with a median age of 13.0 [IQR 11.0–17.0] years]. Informed consent was obtained from each study subject (or legal representatives of the children) prior to being included in the study. The study was approved by the Ethical Committee of the Faculty of Medicine in Pilsen, Charles University (Plzeň).

Oxidative stress parameters ascertained

The following oxidative stress parameters were quantified: plasma malondialdehyde (P-MDA, umol/L), erythrocyte superoxide dismutase (Ery-SOD, U/g Hb), whole blood glutathione peroxidase (WB-GPx, U/g Hb), plasma total antioxidant capacity (P-tAOC, mmol/L), and erythrocyte glutathione (Ery-GSH, mmol/L) using kits from Randox™ (Crumlin, Great Britain), adapted to an automated Hitachi 717 analyser (Roche, Mannheim, Germany) for Ery-SOD, WB-GPx, and P-tAOC or from Oxis Int. (Bonnenie, Marue, France) for Ery-GSH. P-MDA was determined photometrically as TBARS (thiobarbituric acid reactive substances). C-reactive protein (S-CRP, mg/L) was measured as a marker of disease activity using K-assay kit (Labmark, Czech Republic).

DNA damage and DNA repair parameters ascertained

DNA damage and DNA repair capacity were examined with the standard comet assay and its modified version, respectively. DNA single-strand breaks (DNAssb; n/10^9 Da of the DNA) measured by comet assay [22] in peripheral blood lymphocytes isolated from heparinised blood samples were used as a parameter estimating DNA damage. Lymphocytes were isolated by the standard method of centrifugation on a Ficoll density gradient. Viability (assessed by trypan blue exclusion) was typically around 95 %. The cells were set in a layer of 1% low-melting-point agarose on a base layer of 1% standard agarose, and lysed for 1 hour in 2.5 M NaCl, 0.1 M Na_2EDTA, 10 mM Tris-HCl, pH 10, 1% Triton X–100. They were then placed in 0.3 M NaOH, 1 mM Na_2EDTA, pH 13 for 40 min before electrophoresis at <15 °C, 25 V (0.8 V/cm), and approximately 300 mA for 30 min. The comets were visualised by fluorescence microscopy after staining with ethidium bromide. The relative amount of DNA in the tail compared with the head reflects the number of DNA breaks present. The individual's DNA repair capacity (DNArc; n/10^9 Da of the DNA) restoring a standardised oxidative DNA damage was measured by a modified comet assay using cell extracts from the patients' peripheral blood lymphocytes. Lymphocytes isolated as described above were washed in 3x diluted extraction buffer A (45 mM HEPES, 0.4 M KCl, 1 mM EDTA, 0.1 mM dithiothreitol, 10% glycerol, adjusted with KOH to pH 7.8), and centrifuged (700 g, 5 min, 4 °C). As much as possible of the supernatant was removed, the pellet was resuspended by vigorously tapping the tube, and 100 µl buffer A was added for each 10^7 cells. The suspended cells divided into 50 µl aliquots were frozen in liquid nitrogen and stored at –80 °C. Just prior to the assay, a frozen aliquot was thawed, 12 µl 1% Triton X–100 in buffer A was added, and the lysate was centrifuged at 14 000 g for 5 min at 4 °C to remove nuclei and cell debris. The supernatant was mixed with 4 vol reaction buffer B (45 mM HEPES, 0.25 mM EDTA, 2% glycerol, 0.3 mg/ml bovine serum albumin, adjusted to pH 7.8 with KOH) plus 2.5 mM ATP and kept on ice until use. Cultured HeLa cells treated with a photosensitiser Ro 19–8022 were used as target cells with defined amount of oxidative damage. HeLa cells at 2x10^5 per 60 mm dish were incubated overnight in Glasgow-modified MEM (ICN Pharmaceuticals, Basingstoke, UK) with 5% calf serum and 5% foetal calf serum. They were washed twice with ice-cold PBSG (PBS with 0.1% glucose) and 2 ml 0.1 µM photosensitiser Ro 19–8022 (Hoffmann-La Roche, Basel) in PBSG was added to each dish. The cells were irradiated for 2 min on ice, at 330 mm from a 1000 W tungsten halogen lamp. After washing twice with PBSG, the cells were detached by gentle trypsinisation, dispersed by gentle pipetting, split into four aliquots from each dish, and centrifuged for 3 min at 200 g at 4 °C. The pellet of the cells was suspended in 170 µl low melting point agarose (Gibco-BRL) at 37 °C for use in the comet assay. Two 85 µl aliquots of the substrate cells in agarose were placed on a microscope slide that had been precoated with agarose by dipping in a solution of 1% normal electrophoresis grade agarose (Gibco-BRL, Paisley, NI), and drying. This precoating ensures adhesion of agarose gels applied subsequently. Glass coverslips were placed on the gels which were left to set at 4 °C and then placed in lysis solution (2.5 M NaCl, 0.1 M Na_2EDTA, 10 mM Tris made to pH 10 with NaOH, and 1% Triton X–100) for 1 h at 4 °C. The slides were immersed in three changes of buffer B (5 min each). The cell extract (45 µl) prepared from the patient's lymphocytes was added to each gel, covered with a coverslip, and incubated for 45 min at 37 °C in a humid chamber. The slides were then placed in an electrophoresis tank, immersed in 0.3 M NaOH, 1 mM Na_2EDTA (approximately pH 13) for 40 min, before electrophoresis at 25 V (0.8 V/cm), approximately 300 mA, for 30 min. After neutralisation with 0.4 M Tris–HCl, pH 7.5, the comets were stained with 4,6-diaminidino–2-phenylindole (DAPI) and examined by fluorescence microscopy. One hundred comets per gel were classified visually into five categories, according to the intensity of DNA fluorescence in the tail relative to the head, and an overall score for each gel of between 0 and 400 was calculated; this score is linearly related to DNA

break frequency over a wide range of damage. The number of DNA breaks formed in the 8-oxoguanine-containing DNA of target cells by cell extracts was considered as an estimate of the capacity of the examined lymphocytes to initiate the repair of oxidised bases. To express the relationship between the intensity of individual DNA repair and the existing level of oxidative DNA damage, a DNA repair index (DNRI) was calculated according to the formula DNRI = DNArc/DNAssb. The parameters of DNA damage and repair were not available in control adult subjects.

Detection of selected polymorphisms in the RAGE gene

Four single nucleotide polymorphisms (SNPs) in the RAGE gene – the –429T/C and –374T/A (promoter), G82S (exon 3), and 2184A/G (intron 8) – were detected by PCR as previously described [23]. DNA samples were not available in control subjects.

Statistical analysis

The results are expressed as median [interquartile range]. Differences in biochemical parameters were tested using non-parametric tests (Mann-Whitney or Kruskal-Wallis ANOVA). Spearman's correlation coefficients were calculated to assess pair-wise correlation between variables. Bonferroni adjustment for multiple comparisons was used where appropriate. Differences in allele frequencies of SNPs were tested by a two-tail Fisher exact test. STATISTICA for Windows (Statsoft Inc., Tulsa, OK, USA) was used for all standard statistical analyses. P<0.05 was considered statistically significant.

RESULTS

Table 2 shows the values of oxidative stress, DNA damage and DNA repair parameters studied in all subgroups. Comparisons between the two groups were performed by Mann-Whitney test corrected for the total number of comparisons made. After Bonferroni correction, P_{corr} values below 0.006 for comparisons between groups of children (comparison of 8 markers) and below 0.01 for comparisons between groups of adults (comparison of 5 markers) were considered statistically significant to retain the overall $\alpha < 0.05$ significance level. First of all, none of the parameters differed significantly between the adult and children CD patients (P > 0.05, Mann-Whitney). Of all oxidative stress parameters ascertained, solely Ery-SOD differed significantly between CD adult vs. control groups (P_{corr} = 0.0005, Mann-Whitney), being significantly higher in the CD group. None of the parameters differed significantly between the children CD and control groups (P > 0.05, Mann-Whitney). Furthermore, children CD patients exhibited a significant correlation between age and Ery-GSH and P-MDA (r = –0.6, P = 0.005 and r = 0.5, P = 0.02, respectively, Spearman), while DNA damage and the remaining oxidative stress markers did not correlate (P > 0.05, Spearman). No such correlation was ascertained in adult CD patients. Adult CD patients exhibited a significant correlation between disease duration and DNA repair (DNArc, r = 0.48, P = 0.039, Spearman), while DNA damage (DNAssb) and oxidative stress parameters did not correlate (P > 0.05, Spearman). No such correlation was ascertained in children with CD.

Analysis of the disease activity – characterised by either CDAI or PCDAI – revealed a significant negative correlation between both CDAI and PCDAI and P-tAOC in children (r = –0.5, P = 0.02 and r = –0.53, P = 0.013, respectively, Spearman) together with a negative correlation between both CDAI and PCDAI and WB-GPx (r = –0.5, P = 0.03 and r = –0.51, P = 0.018, respectively, Spearman), while no correlation between disease activity and any of the parameters studied was found in adults.

Comparison of CD patients with and without history of complications (i.e. abscess, fistula or stenosis) identified statistically significant differences in Ery-GSH (2.14 [1.98–2.3] vs. 2.29 [2.11–2.71] respectively, P = 0.05, Mann-Whitney) and P-MDA (1.86 [1.47–2.23] vs. 1.48 [1.28–1.82], respectively, P = 0.015, Mann-Whitney).

Finally, there was no statistically significant difference in the allele frequencies of any of the four SNPs in the RAGE gene analysed between the adult or children groups (P < 0.05, Fisher exact test, data not shown). Furthermore, no relationships between the carrier state of any of the four RAGE SNPs and oxidative stress, DNA damage or DNA repair parameters analysed were ascertained (P > 0.05, Kruskal-Wallis ANOVA, data not shown).

DISCUSSION

Although oxidative stress and DNA damage are believed to play important roles in the pathogenesis of CD, the literature on this topic is scarce so far. The majority of published studies involved adults. In a recent study by Beltran, peripheral immune cells of CD patients were shown to have increased activity of SOD and production of H_2O_2, while catalase activity was decreased compared to the immune cells of healthy control subjects; similarly, the parameters of oxidative damage (P-MDA and 8-oxo–2'-deoxyguanosine) were elevated [24]. In our present study we investigated the relationships between the extent of oxidative stress and DNA damage/repair, disease severity, and selected common genetic variants in the RAGE gene in CD patients. We found significantly higher Ery-SOD levels in CD adult patients compared to adult healthy controls. This is in agreement with the formerly mentioned study and also with the results published by Dincer et al [25]. However, while they were able to detect higher WB-GPx levels and

increased DNA damage in CD patients [25], this was not the case in our study. Additionally, we found a significant positive correlation between disease duration and DNA repair in adult CD patients. This might suggest the compensatory increased capacity to repair oxidative and genotoxic damage in the situation of long-term inflammatory disease.

Koutroubakis et al. [26] found a significantly lower level of P-tAOC in CD patients compared to control subjects, which was not ubiquitously confirmed by our study. However, we showed that the disease activity in children (characterised by either CDAI or PCDAI) correlated negatively with P-tAOC. Furthermore, we found a negative correlation between WB-GPx and PCDAI in CD children. This finding shows that in childhood subjects with more severe CD exhibit lower markers of antioxidant defence suggesting failure of compensatory antioxidant defence with advancing disease. Finally, a comparison of the parameters between subjects with and without history of complications showed significant differences in Ery-GSH and P-MDA levels. Apparently, subjects with personal history of CD complications are characterised by a higher rate of oxidative stress (P-MDA), while the level of non-enzymatic antioxidant Ery-GSH is decreased.

To our knowledge, genetic variability in the gene encoding for RAGE has not been studied in relation to CD so far. We did not find any relationship between gene variants and presence of disease or relevant biochemical parameters. Therefore, we can probably exclude any strong effect of RAGE gene variants on CD course. However, considering the low number of subjects in this study, the statistical power was unfortunately too low to detect moderate to mild genetic effects.

In summary, the results obtained in the present study of CD patients of variable age primarily demonstrate that intestinal inflammation is reflected in selected circulating markers of oxidative and genotoxic stress. Second, the impact of the disease seems to be different in adults and child patients – while selected antioxidant (Ery-SOD) and DNA repair parameters (DNArc) are up-regulated in adult CD patients, advancing disease severity is accompanied by decreased selected antioxidant parameters in children (P-tAOC and WB-GPx). Finally, the presence of CD complications is reflected in increased markers of oxidative damage (P-MDA) and decreased cellular antioxidant defence (Ery-GSH). However, we have not confirmed universal involvement of DNA damage and subsequent repair in the CD course. As regards the role of RAGE genetic variability, signalling, and contribution of endo- and exogenous RAGE ligands (calgranulins, Maillard reaction products or AGEs) as potentially aggravating factors of CD progression, a further well-powered study is required.

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