Faculty of Medicine • Masaryk University • Brno • Czech Republic

PROCEEDINGS

SYMPOSIUM

THE IMPORTANCE OF CHRONOBIOLOGY IN DIAGNOSING AND THERAPY OF INTERNAL DISEASES

Dedicated to the 60th Anniversary of Professor Jarmila Siegelová

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January 10, 2002

The Symposium takes place under the auspices of

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CONTENS

PERSONAL REPORT

Prof. MUDr. Jarmila Siegelová, DrSc.,
a woman celebrating her 60th birthday

It sounds almost unlikely but the date in her birth certificate is inexorably true. At the beginning of January 2002 Prof. MUDr. Jarmila Siegelová, DrSc., head physician of the Clinic of Functional Diagnostics and Rehabilitation at St. Ann Teaching Hospital and the Medical Faculty of the Masaryk University Brno, celebrates her 60th birthday. Many people can´t believe it. Especially those who at present have the honour of close co-operation with Prof. Siegelová but also those with whom she co-operated in past. In spite of all troubles prepared for her in past by her life her optimism, she was spreading in past and still spreads around her, makes her seem to be a happy and successful person.

Since her graduation at Masaryk University Brno in 1965 professor Siegelová has gone through a long journey of scientific work characterised by immense diligence, a journey of overcoming numerous difficulties and also of important scientific successes. For her first scientific works she used experimental animals and, as characteristic for her in her further scientific work, she decided for methodically difficult experiments with stimulation and reading action potentials of nerve fibres, when examining the role of splanchnic nerves in regulation of breathing. She many times later returned to the subject of breathing regulation, however, for a change in a healthy and an ill person. She habilitated with this subject and become a member of the International Society of Pathophysiology of Breathing and she was repeatedly invited to have lectures at international scientific conferences. Her doctoral thesis (defended in 1990) referred to the regulation of breathing in a healthy person and in selected pathophysiological conditions. In 1997 she was appointed as a professor in the field of normal and pathological physiology.

Professor Siegelová has very right and very soon understood the necessity of international co-operation where she could use not only her very good knowledge of foreign languages but also her great talent for organising. She become a member of an important international team which arose around Prof. Halberg, the director of Chronobiological Laboratories of the University of Minnesota (USA), one of the founders of chronobiology as a scientific branch. That way she has become a co-author of a range of scientific works of an international importance. She particularly concentrated on that part of chronobiology which referred to adaptation of therapy to individual differences of people, sometimes called "individually tailored therapy". Accordingly, it concerned practical application of chronobiology to treatment in particular. On the others side, as a leading member of this group of authors she is the author of an important discovery which is of a basic biological importance. Her main merit is a discovery of a week rhythm in circulatory quantities which results from natural regularities and not from evolution of the society. She has found a 7-day fluctuation of blood pressure and

pulse rate in new-borns at the Teaching Hospital Brno which is synchronised by birth and is independent of week days. That means we have the week programmed in us and development of society in various cultures where the 7th day is a holiday is probably determined by this biological phenomenon. The result was also confirmed in new-borns in Minnesota and Spanish La Corona. The main focus of her work is the study of circulatory rhythms in people with hypertonia. She was present at the beginnings of 24 hour monitoring of blood pressure in people with hypertonia in our republic when as a leading author in 1993 published the study where she compared various evaluation methods of blood pressure values in 19 treated and not treated people with hypertonia. But of an international importance are works addressing continuous monitoring of blood pressure lasting several days, where Minnesota group, of which working team she is a member, has world primacy. Her international co-operation is not only limited by USA. Joined publications demonstrate joined works with Lariboisiere Hospital in Paris and with University in Graz. Among their fellow workers are as famous personalities as Prof. Martineaud from France and Prof. Kenner from Austria.

Professor Siegelová is of course a member of a range of international scientific societies such as ISH - International Society of Hypertension, European Respiratory Society, Association of French Physiologists - Société de physiologie, NY. Academy of Sciences. At present she governs the Clinic of Functional Diagnostics and Rehabilitation of the Medical Faculty and the Masaryk University Brno.

At present professor Siegelová concentrates on problems of pathogenesis and treatment of essential hypertension and issues of chronobiology of blood pressure and heart rate. She is known for her bright debating and uncharitable critic of iniquities. She has never cribbed scientific knowledge of others nor she sponged upon publications of the others. On the contrary she has generously and disinterestedly given from her own hits without demand on authorship. She has never pretended to know everything and has been happy to accept advices from any of her colleges.

Her love for nature, fiction, and all her out of work activities are stable part of her life optimism which is also an important part of her impact to patients and colleges and lead to a feeling of sympathy and safety. She loves upstanding, tough, and brave people, she can´t stand hypochondriacs, hysterics, and whimpers. She is very interested in fates of people and never forgets to comfort the ill with a word, glance, smile.

All the best wishes for many other active years.

Prof. MUDr. Bohumil Fišer, CSc.,
Minister of Health of the Czech Republic and the chief of the Physiological
Institute of the Faculty of Medicine MU Brno
Komenského nám. 2
662 43 Brno

CHRONOMICS COMPLEMENT GENETICS IN BRNO

WHAT JOHANN GREGOR MENDEL WISHED, JARMILKA SIEGELOVA ACCOMPLISHED: BROADENING SYSTEM TIMES AND TRANSDISCIPLINARY TIME HORIZONS*

Franz Halberg, Germaine Cornélissen, Pavel Prikryl •, George Katinas, Jiri Dusek•, Pavel Homolka•, Zdenek Karpisek*, Robert P. Sonkowsky, Othild Schwartzkopff, Bohumil Fiser•‡,
Jarmila Siegelova• and the International BIOCOS Project Team§

University of Minnesota, Minneapolis, Minnesota USA
•Masaryk University, Brno, Czech Republic
‡Ministry of Health, Prague, Czech Republic

Address correspondence to:

Prof. Franz Halberg, M.D.
Director, Halberg Chronobiology Center
University of Minnesota
715 Mayo Building
Mayo Mail Code 8609
420 Delaware St. S.E.
Minneapolis, MN 55455, USA
PHONE +1 (612) 624.6976, 624.3144 OR 626.5404; +1 (651) 454.6334 (mornings)
FAX +1 (612) 624.9989 · E-MAIL halbe001@tc.umn.edu

*Invited lecture on chronomics at Masaryk University, Brno, Czech Republic.

Key words: chronomics, chronobiology, history of science, history of statistics, time series, Wolf's relative sunspot numbers

§Armenia: Noubar Aslanian, Institute of Cardiology, Yerevan.
Austria: Manfred Herold, University of Innsbruck.
Belgium: Christian Muller, Olga Quadens, Aerospace Institute, Brussels; Alain Delcourt and Guy Toussaint, private practice physicians, Brussels.
Brazil: Ana Amélia Benedito Silva, Nelson Marques, Luiz Menna Barreto, University of Sao Paulo.
Bulgaria: Nadejda Madjirova, Department of Psychiatry, Medical University, Plovdiv.
China: Zhengrong Wang, West China University of Medical Sciences, Chengdu; Ziyan Zhao, Shandong Academy of Medical Sciences, Jinan.
Czech Republic: Bohumil Fiser, Jarmila Siegelova, Jiri Dusek, Pavel Homolka, Muhammad Al-Kubati, Pavel Prikryl, Masaryk University, Brno.
Germany: Sigrid Berger, Max-Planck-Institute für Zellbiologie, Ladenburg; Armin Grafe, GFZ Potsdam, Niemegk; Karl Hecht, Charité Hospital, Humboldt University, Berlin; Max Halhuber, Bad Berleburg; Thomas Müller-Bohn, Pharm.D., Süsel-Holstein.
India: R.B. Singh, Medical Hospital and Research Centre, Centre for Nutrition and Heart Research, Moradabad; R.K. Singh, Department of Biochemistry, King George's Medical College, Lucknow.
Italy: Franca Carandente, Department of Chronobiology, and Cristina Maggioni, Department of Obstetrics, University of Milan; Federico Perfetto, Roberto Tarquini, Riccardo Livi, Department of Medicine; Giorgio Mello, Department of Obstetrics, and Giancarlo Mainardi, Department of Pediatrics, University of Florence; Andrea Galvagno, Maurizio Montalbini, Pioneer Researches, Ancona.
Japan: Kuniaki Otsuka, Yoshihiko Watanabe, Department of Medicine, Tokyo Women's Medical University, Tokyo; Terukazu Kawasaki and Keiko Uezono, Institute of Health Sciences, Kyushu University, Fukuoka; Yuji Kumagai, Jichi Medical School, Tochigi.
Mexico: Salvador Sánchez de la Pena, Instituto Mexicano del Seguro Social, Mexico City; Clicerio Gonzalez, Centro de Diabetes Mexico City; Maria Luisa Fanjul Moles, L. Neurofisiologia Comparada, Coyoacan.
Nigeria: S.O. Ogunade, Obafemi Awolowo University, Ile-Ife.
Norway: Andi Weydahl, Finnmark College, Alta.
Romania: Ion Baciu, Professor and Head of Physiology, Oradea University, Oradea.
Russia: Mikhail Blank and Olga Denisova, N.N. Petrov Oncology Research Institute, Ministry of Health, and Mikhail Denisov, St. Petersburg Special Mental Hospital, St. Petersburg; Tamara K. Breus, Space Research Institute, Moscow; Maria Mitish, Elena V. Syutkina, Tatiana Turti, Galina V. Yatsyk, Institute of Pediatrics, Academy of Medical Sciences, Moscow; Anatoly Masalov, Lebedev Physics Institute, Moscow; Rina M. Zaslavskaya and Mikhail M. Teibloom, Moscow Hospital #60, Moscow.
Slovakia: Miroslav Mikulecky Sr., Comenius University, Bratislava; Alexander Kreze, Lubochna.
South Africa: Mary-Jane Thaela, Animal Nutrition and Animal Products Institute, Irene.
Spain: Leopoldo Garcia Alonso, Hospital Materno-Infantil 'Teresa Herrera', La Coruna; Francisco del Pozo Guerrero, Maria Teresa Arredondo Waldmeyer and

Maria José Rodriguez Fernandez, Department of Telecommunications, Universidad Politecnico de Madrid; Miguel Revilla, Department of Mathematics and Computer Science, University of Valladolid.
Sweden: Lennart Wetterberg, Karolinska Institute, Stockholm.
Ukraine: Anatoly Delyukov, Senior Scientist, Institute of Physics, Ukrainian Academy of Sciences, Kiev; Yuri Gorgo, Department of Human and Animal Physiology, Faculty of Biology, Kiev National Taras Shevchenko University, Kiev.
UK: Douglas Wilson, ADAS Bioengineering Unit, Cheltenham, England; Sam K. Arthur, Gastroenterology Department, Queen Mary and Westfield College, University of London, England; Hugh Simpson, Department of Surgery, University of Glasgow, Scotland.
USA: Germaine Cornélissen, Franz Halberg, Julia Halberg, Chronobiology Laboratories; Dana Johnson, Department of Pediatrics; Christopher Bingham, Douglas Hawkins, Department of Applied Statistics; Jim Holte, Department of Electrical Engineering; Robert Patterson, Bakken Medical Instrumentation and Device Laboratory, University of Minnesota, Minneapolis, Minnesota; Mark Engebretson, Department of Space Physics, Augsburg College, Minneapolis, Minnesota; Henry Fink, Richard Kopher and Joseph Rigatuso, HealthPartners, St. Paul, Minnesota; Earl E. Bakken, North Hawaii Community Hospital, Kamuela, Hawaii; Patrick Delmore, Medtronic Inc., Minneapolis, Minnesota; Francine Halberg, Marin General Hospital, Greenbrae, California.

ABSTRACT

The mapping of time structures, chronomes, constitutes an endeavor spawned by chronobiology: chronomics. This cartography in time shows signatures on the surface of the earth of the about 21-year (circadidecadal or circavigintunennian) Hale cycle in the polarity of sunspots. Circadidecadals complement about 10.5-year (circadecadal or circaundecennian) and about 50-year (circaquindecadal or circasemicentennian) cycles, also accumulating in life on the earth's surface. We append a glossary of these and other cycles, the names being coined in the light of approximate cycle length. Many of the unselected, seemingly ubiquitous infra-annual cycles, as Miroslav Mikulecky calls them, are inferentially statistically validated with their uncertainties. These findings are transdisciplinary, in view of their broad representation and critical importance in the biosphere. Suggestions of mechanisms are derived from an analytical statistical documentation of characteristics with superposed epochs (with control epochs) and/or superposed cycles and other "remove-and-replace" approaches. These approaches use the spontaneously changing presence or absence of an environmental, cyclic or other factor for the study of any corresponding changes in the biosphere. Hints of putative partial endogenicity stem from non-overlapping 95% confidence intervals of near-matching biospherical and physical periods in variables that were linked by the foregoing methods. Solar flares, storms in the interplanetary magnetic field, major geomagnetic and/or cosmic ray disturbances, that all exhibit cycles, may play a role as potential synchronizers of multidecadal cycles built into populations. They may also elicit, as single events, sequences of rhythmically recurring, very different effects, i.e., feedsideward intermodulations, to be revealed by a phase-response curve.

In the light of the foregoing, we illustrate the indispensability of the mapping of rhythm characteristics in broader structures, chronomes, along several or all available different time scales. The results of this mapping, chronomics, can be used to plan the system time of future studies and to interpret results in the context of a broader time horizon, e.g., in health care or basic science. This mapping is done in Brno, the birthplace of genetics. We draw a parallel between the mapping of the genome, genomics, spawned by genetics, and that of chronomics, an offshoot of chronobiology. We present results from a cooperative cartography of ~10, ~20 and ~50-year rhythms in the context of a broad endeavor concerned with the BIOsphere and the COSmos, the BIOCOS project. The participants in this project are our co-authors worldwide, beyond Brno and Minneapolis, and are co-felicitators herein on the 60th birthday of Jarmila Siegelova, whose cartography of human blood pressure and heart rate around the clock and along the week may provide the evidence for those influences that Mendel sought in meteorology and climatology.

INTRODUCTION

A very broad concern for diverse changes with time led Herbert Hörz to philosophically coin the terms "system time" and "time horizon" (1). We here apply these terms empirically from the viewpoint of chronomics, the cartography of time structures, i.e., chronomes (2). Empirically, the time scale, along which we intend to sample in a given observational, clinical or experimental test or other study,

may be called the system time. The complementary time horizon in turn includes all retrievable, inferentially statistically analyzed pertinent prior information. The time horizon thus consists of the rhythm, chaos and/or trend characteristics and of their changes with time in the variable(s) under consideration. The durations and temporal locations of the timepoints at which earlier sampling was done, have to be specified, of course, as system times and their sum constitutes the time horizon, enlarged by each addition. These characteristics become useful only after as many as possible observations made with all previously used system times are summarized as chronomes. The chronobiologic aspects of parts of ontogeny and phylogeny are being documented for one or a few variables in the rat, piglet and human newborn. A synthesis of these different results shows an initial prominence of circaseptans, ascertained in a comparative physiological time horizon, which is as yet limited to early human and other post-uterine life and to early stages of crayfish development. One can then add that circaseptans can also be more prominent than circadians in *Acetabularia acetabulum*, a unicellular plant. By contrast, in as yet very limited time series of observations on E. coli and cyanobacteria, circaseptans, albeit present, have a smaller amplitude as compared to circadians.

Along such lines, much more study is required to attempt to track evolution chronobiologically. Incomplete information, again restricted to a few test pilots, is available for the case of the human heart rate, blood pressure and their variabilities. Eventually, all biological variables will require systematic mapping, whether for tracking an internal integrative as well as external adaptive Darwinian evolution on the one hand (3), and for reference values in health and disease on the other hand (4).

An international project on the BIOsphere and the COSmos, BIOCOS, has enlarged the transdisciplinary time horizon and torn down barriers for students of physiology in general, including the circulation. Archival studies complement physiological monitoring to reveal multidecadal changes in the chronomes not only of blood pressure and heart rate, but also in the incidences of disease such as strokes and myocardial infarctions. These latter conditions carry circaquindecadal and circadecadal cyclic signatures, respectively.

We illustrate the need for mapping chronomes by showing what could happen when any mapping already accomplished remains ignored (albeit it should constitute a useful time horizon). We also indicate the putative utility of a time horizon to health care and basic science, once the mapping proceeds, for instance for the detection of CHAT (short for circadian **h**yper-**a**mplitude-**t**ension). The long-term major public health task of picking up in time the elevated risk of vascular disease in adults can be documented. It is to be complemented eventually in the human newborn at birth, if not at conception. We seek disease risk detection in the physiological range, for the clinics an endeavor requiring the collection of reference values. Work toward this goal provides, as a dividend, a broad time horizon, emerging from a network of international cooperation; we may reap ample dividends first and foremost for the interpretation of studies along the system time of long-term lifetime monitoring, implemented by a test pilot by self-measurement of 11 variables about 5 times on most days for over 3 decades and by another colleague automatically every 30 minutes for well over a decade for blood pressure and heart rate. Systematic monitoring leads to pre-habilitation for

eventually replacing or reducing the need for rehabilitation after catastrophic disease has occurred (4).

From genetics to chronomes in Brno

The signature of Johann Gregor Mendel, the founder of genetics, is present, beyond his mid-19th-century Brno, throughout the world of genetics and now genomics, and is currently making headlines with stem-cell research and human cloning. Also present in the contemporary mind of investigators involved in BIOCOS is the mapping of time structures in the human circulation and around us, the chronomes in the organism and the environment. This mapping endeavor, associated in Brno and beyond with the personality of Jarmila Siegelova, and the title of this presentation reflect the thread leading from Johann Gregor to Jarmilka, their intellectual *Wahlverschandtschaft*. The pea patch in front of the Brno abbey, cultivated by Mendel, was not his only interest (5). Mendel published only two papers on plant hybridization; he published much more extensively in the field of meteorology. In private correspondence, Mendel was concerned about the "telluric and cosmic influences" (6) that enable some but not most other hybrids to survive severe environmental conditions. Could he have anticipated chronomics (2) more than chronobiology (7), the study of the built-in mechanisms of human time structures, documented by studies, among others, on twins reared apart to be genetically based (8)?

Mendel would certainly enjoy the documentation, by the BIOCOS team, that genomes have incorporated more and more of the cycles of the very environment in which they evolved, not only by an adaptive natural selection but also by an integrative evolution (3). Some of the evidence is obtained as circadian amplitudes from the fit of a 24-hour cosine curve to the heart rates of twins reared apart (not here shown). When these amplitudes are analyzed by intraclass correlation coefficients, they reveal heritability, specified in Figure 1 (8). Chronomics, the mapping of the expression of genes in the form of chronomes, consisting of multifrequency rhythms in us and their interactions with those outside us, is well under way in Brno to answer whether the results of Figure 2, in the otherwise neglected physiological range, are a curiosity of certain Asians or also apply to some extent to other populations, including Caucasians. The scope of these Figure 2 results is being checked at Brno's St. Anna Hospital by Pavel Homolka with 7-day/24-hour monitoring. As a start, over 100 such 7-day series have already accumulated. The 24-hour profile is a rather false gold standard, a fact documented elsewhere (9, 10).

Need to separate transient 24-h CHAT from 7-day CHAT

In a seemingly well woman in her 60s (CH), the circadian amplitude of systolic blood pressure was only transiently elevated in the first profile as shown by the first dot in Figure 3. For the ensuing 6 years, the usually longer than 1-week profiles were all acceptable in terms of the circadian amplitude. In this case, the initial elevation of the circadian amplitude was associated, on different days, with trouble at work, bad memories and grief, all recorded prospectively in a diary, as described elsewhere (10). The patient did have intermittent systolic and diastolic circadian hyper-amplitude-tension, CHAT. Her case was a transient **24-hour** CHAT, not only initially but also 6 years later, yet not for a sufficient time to

diagnose "**7-day** CHAT" based on a 7-day or, in such cases of transient or **24-hour** CHAT, based on a much longer record. Initially, CH had very high blood pressure for 5 consecutive days, but again not for the rest of over 20 days of the first monitoring, so that in long profiles, except for a very psychologically taxing (emotional) event (shown by a vertical arrow), her blood pressure MESOR, a chronome-adjusted mean (not shown), was below the upper chronobiologic limit. (The last profile, however, after 6 years had an elevated MESOR, as confirmed by the time course of indices other than the MESOR such as the percent time elevation and the hyperbaric index, which were all elevated only transiently, in association with emotions or at the end of the 6-year monitoring span.)

Had one acted based on the 5 days of initial MESOR-hypertension and treated for the intervening 6 years, one might have had side effects and unnecessary expense. In view of the chronobiologic diagnosis, the patient remained untreated and was doing well six years later. Other similar cases (9-12) include that of a cardiologist who had very high pressures for 48 hours but an overall acceptable 7-day profile not only to start with, but also 7 years later at the age of 93 (12). In the interim he was untreated, but was doing very well. Nonetheless, such long intervals between monitoring, as in the case of the cardiologist, are not recommended. The minimal system time in these cases was the 7-day/24-hour monitoring, preferably on a yearly or, if indicated, by a transient abnormality more often, as in the case of CH. The real time horizons will be the lifelong perspectives, provided by such test pilots, a majority of them physicians themselves (12-14). But there is another side to this story. Only controlled large chronobiologic trials, preferably with timed treatment, can decide at what level non-drug or drug treatment is indicated.

Building further on quicksand

A recent article by Vasan et al. based on the Framingham Study suggests that high normal values should be treated (15). Before one adheres to such a recommendation, a point mentioned by Panza in an editorial (16) on this article (15) concerns the lack of reliability of the conventional diagnosis. The editorial, entitled "High-normal blood pressure - more 'high' than 'normal'", comments on

> the risk of cardiovascular disease among a subgroup of the Framingham Heart Study population without hypertension at base line, classified according to their base-line blood pressure. The authors found that the participants with high-normal blood pressure (systolic pressure of 130 to 139 mm Hg, diastolic pressure of 85 to 89 mm Hg, or both) had higher rates of cardiovascular events than those with optimal blood pressure (defined as systolic pressure of less than 120 mm Hg and diastolic pressure of less than 80 mm Hg). These findings lend further credence to the theory that high-normal blood pressure must be categorized differently from normal or optimal blood pressure.
>
> Certain aspects of the study affect its clinical relevance. First, blood-pressure measurements were obtained at single time points. Given the variability of blood pressure, particularly in this borderline range, these measurements may not represent the average value during daily activities. (16)

The realization is overdue that the "high normals" were so diagnosed by virtue of a snapshot on a roller coaster with 40% or more uncertainty (17). This uncertainty must be eliminated before solid recommendations for the treatment of individual patients can be made. The uncertainties even of 24-hour or longer profiles, including 5-day profiles, have been demonstrated (9). A spontaneous remark by an editor on a paper published by us in his journal seems pertinent (18; cf. 19):

> Talking about "blood pressure" as a single figure is similar to knowing the average height of a mountain range: an interesting statistic, but completely useless to a pilot trying to make it through a mountain pass alive. Realistically, we need to consider not merely the mean [average] stress on an aging vascular endothelial cell, but the "peaks" that it has to "fly over" as well. Aging vessels are--to an extent--the end result of such stresses. Halberg et al. suggest that many patients may be apparently normotensive [with normal blood pressure], yet (because of circadian peaks in blood pressure) have the catastrophic risks of any other severely hypertensive patient. They recommend that [medical practitioners] avoid "flying blind" and begin to measure peak pressures more accurately if we are to avoid disaster.

Chronobiologic guidelines for archivization

Bohumil Fiser et al. have greatly broadened the time horizon in archivization by showing a 50-year cycle in stroke incidence in the Czech Republic as in Minnesota (20, 21). The proper coding for medical chronomics, relating to natality, morbidity and mortality, is overdue and is an urgent governmental task. Like the maintenance of clean air, clean water, and clean and safe streets, the surveillance by monitoring and proper archivization could become a service on the immediately practical side (22); but perhaps, it may have to be privatized if people do not value what is offered free of cost.

Detour via Mendel and Siegelova from heliobiology to chronoastrobiology

The physico-chemical environment on earth and beyond has a number of more or less periodic features that may be classified as photic (or more broadly as obvious and sensed environmental temperature effects included) and non-photic (hidden; subtle). The sun and earth are both magnets. Helio- and geomagnetics contribute with galactic cosmic rays their share of non-photic effects, that can be traced by auroras, sunspots and recently by recordings not only on earth but also from vehicles in space. The photic effects are now known to be coded in the genome as circadian and circannual systems (7). Among non-photic environmental effects, we have found first a near-week in the environment (23), confirmed by physicists (24), shown in environmental geophysical activity in Figure 4. Germaine Cornélissen has also just found proof from studies on twins that the week as well is anchored in our genes (25). Figure 5 shows the geomagnetic half-year, and non-photic biological associations are also mapped with about 10.5-year and other components of solar activity, some shown in the time domain in Figure 6, and in the frequency domain in Figures 7. We can summarize these and other results from mapping rhythms into rules of procedure based on the proposition that during a billion or so years on or in earth, life had to integrate into a cyclic environment not only by adjusting to cycles but also by coding them as features of

organismic and environmental adaptation (26) or integration in genomes (3). The finding by others (27-29) of a half-year component in the geomagnetic index Kp led us first to show its particular prominence in a phase-weighted cosinor spectrum (30), Figure 5, and further to the finding of a circasemiannual near-matching periodicity in a vast number of biological phenomena. While the biological week led to the geophysical week, the information on the half-year, emphasized to us by the geophysicist Armin Grave (27), led us to the detection of a circasemiannual component in many variables of the biosphere and to an amplification of the proposition of an internal evolution (3, 31).

Results from mapping rhythms in chronomes

More generally we propose:

1. Any old or newly observed periodicity in the physico-chemical or socio-ecological environment should prompt the search for a near-match in biology as a putative synchronizer or influencer and/or, what seems at least equally likely, as a feature built into organisms. Thus, we find circasemiannuals in the birth rate at high latitudes (32), in body weight and height at birth (33. 34), in gain in weight and height during the first 15 months of life (35). There is a cross-spectral coherence coefficient with K_p of 0.74 at 5.91 months, away from a spectral peak, in the blood pressure and heart rate of a clinically healthy man who self-measured these and 9 other variables for over 3 decades (>.5 million values) (21). There is further a half-yearly component in the circulating melatonin of human pregnancies with intrauterine growth retardation (36) (but not in clinically healthy pregnancies). At middle latitudes, at a solar activity minimum, but not in the ascending stage of solar activity, human circulating melatonin exhibits a circasemiannual component by night (37) and a circannual during the daylight hours, whereas melatonin shows a circasemiannual component at noon at high latitudes (37; cf. 38). In Figure 10, test 2 (for a circasemiannual component in nightly melatonin of subjects at middle latitude) works at solar minimum but not in the ascending stage of solar activity. Thus, frequencies interact and some intermodulations constitute new testable items to be fitted into an ever broader picture puzzle that may turn into novel chronome maps. Circasemiannuals further characterize the vasopressin-containing nuclei of the human hypothalamus (39), the incidence of hallucinations (40) and most prominently the incidence of status epilepticus, Figure 11, during a 3-year span of intensive magnetic activity (23).

2. When we find a biologically new periodicity, previously regarded as purely societal (41, 42), we have to search for a near-match in the natural physical environment. If there is not a very prominent near-match in the environment, the new periodicity such as that of a biological free-running week found in the urinary 17-ketosteroid excretion of a healthy man (43), we may speculate that the week may stem from one that was present prominently in an ancient past, when rhythmicity, such as that of a week or of a half-week existed or the periodicity came about in response to internal needs, as a feature of an internal integrative evolution (3), complementing a Darwinian adaptive evolution by natural selection (26). The latter internal mechanism was the original hypothesis (31) until we found an average about 6.75-day periodicity in a 59-year record of K_p, Figure 4, which was subsequently corroborated by a similar near (but not exactly) matching component in 110 years of data on another geomagnetic index, aa (24), and now by

our analysis of 134 years of data on aa; the amplitude of the near-match of the biological week about 130 years ago was weak, as it is now (44, 45). As originally postulated (3, 31), internal conditions may still have played the major role in acquiring a genetically anchored week (31). Unquestionably, however, on the average there is a near, but not exactly 7-day peaklet in geomagnetic pulsations also recorded in the presumably less polluted Antarctic by a stand-alone magnetometer, 610 km from the nearest habitat; albeit small, it has the largest amplitude in that spectral region (25).

3. Biological rhythms without an environmental near match may point to the disappearance in the course of life's development on earth, of corresponding near-matching natural physical environmental cycles. Like the foregoing ones, this possibility has to be qualified by the circumstance of an internal evolution, postulating that some periodicities came about for matters of internal coordination, exclusively or at least primarily for this reason. Of course, matters of sheer chance by random mutations cannot be excluded. The information of today may have to be qualified tomorrow.

A current challenge is the search for a possible environmental counterpart for about 8-hourly rhythms in vasoactive substances such as norepinephrine and epinephrine (only on an equidistant isocaloric diet thus far) (46) and (under ordinary conditions but not invariably) in the case of endothelin-1 in the human circulation (47-49) and in the population density of endotheliocytes in mouse ear (50) and in venous human (51) and portal pig blood melatonin (52). Whether an 8-hour periodicity in endothelin is a key connecting rhythms and chaos pertains to the search for a similar periodicity in other vasoactive substances under ordinary conditions, such as in the case of substance P. It will take further studies to see whether the complementary aspects of chronos and chaos in our lives intersect at about-circaoctohoran features as a mechanism in its own right, coded in our genes, or whether 8-hour rhythms merely represent, as the third harmonic, the waveform, of a fundamental circadian system physiology.

4. In their growth and development, individuals and populations of human beings and other multi- or unicellulars, eu, or prokaryotes, have already proved to be living fossils that may replay in their rhythmic dynamics during ontogeny, the sequences that occurred during the development of life (23, 53). Crayfish (54) may be a better subject of study in this context than human beings (55, 56), rats (57) or pigs (58), for the investigation of the biological week, since a circaseptan is present with an amplitude larger than that of the circadian in crayfish locomotor activity at 6 months of age.

5. For any endeavor in any clinical and/or purely scientific context, maps from preferably automatic monitoring along more than the time scale chosen for recording (i.e, the system chronome) are desirable. Monitoring and analyses are best planned by considering all complementary chronome information (chronome horizon); based on the latter, the T (the duration of recording providing the number of replications) and the Δt (the interval(s) between consecutive observations) (for data density) are estimated. In spatial travel, we may use in addition to our city map, a large world map, and after arrival in a new location, again a new city map. Similarly, we have mapped in time and space, among many other aspects of the human brain, religious motivation in Alaska (59) and then in 103 countries around the world (60), Figure 12. East and west of Greenwich and

north and south of the equator, in nations near the equator and in those closer to the poles, we have found about 21-year signatures of the Hale cycle in this presumably brain-related index of religious activity, summarized in Figure 12. Figure 13 shows a lesser 21- and a major about 50-year cycle in homicides. More 50-year cycles are summarized in Figure 14, including one in international battles over the past 2,556 years. Chronome mapping complements geographic mapping in dealing with the health or disease of individuals or societies. Chronomics, rather than single-sample- or 24-hour-based spotchecks, are best combined with a broader time horizon, wherein chronomics complements genomics and proteomics, even as we trace ancestors in science (61) or in our evolution or make preparation with safeguards, as we venture into space.

Confounding nonsense becomes new information with chronomics

The role of multidecadal rhythms is illustrated by the example of the excretion of metabolites of steroidal hormones (17-KS); over several years, a decrease with age ($P<0.001$) was demonstrated, Figure 14a; during the ensuing several years, in the same subject there was an increase with age ($P<0.001$), Figure 14b. When all data over 15 years were used, it was clear that one was dealing with a spontaneous about 10-year cycle ($P<0.001$), Figure 14c. In different stages of this cycle, there were spans of positive, Figure 14b, negative, Figure 14a, and no correlations with age, the first, third and fifth section of Figure 14d. Nonsense correlations may also account for much controversy attempting to relate biological variables to environmental ones, such as Wolf's relative sunspot numbers, WN, and the geomagnetic activity index, K_p, both shown in Figure 14e. A nonsense correlation thus occurs not only between steroids and age, Figures 14 a, b and d, but also between steroids and helio- or geomagnetic indices, Figure 14 f (left), if cycles shown on the right of this figure are ignored. These results can be generalized. Unmapped biological cycles corresponding in length to those of solar activity will be confounders with ignorance hidden by the term "secularity". Confounding and irreproducible results are inescapable when rhythmic functions are involved yet ignored. This prompts the rhetorical question: What variables are not rhythmic?

On the positive side, Figure 14g shows the time relations of solar activity to steroid excretion with the right timing (a small lead in phase) for a putative role played by the sun in affecting hormones that relate to resistance to disease (7, 21, 43, 53). This hint from both the periods and the phases involved is strengthened by a cross-spectral coherence between the two variables, 17KS and WN (details given below curves in Figure 14g). Figure 14h is a partial chronome map of periods showing rhythms with many frequencies modulating a prominent circadian component (cf. 7). This and similar information can be usefully considered in all investigations that involve studies with system times corresponding to just one component in the spectrum of a given variable investigated, such as steroid excretion and innumerable others. What may appear to be focused research on the circadian system may actually be subject to a host of intermodulations (see Figure 10).

Multidecadal rhythms may relate to the non-photic environment, a possibility suggested first by a scrutiny of mechanisms by superposed epochs with controlled epochs, by superposed cycles and by following spontaneous changes in solar or geomagnetic activity, for associations in the biosphere when certain components in

the spectrum of the sun are present, or absent, i.e., by an approach comparable in endocrinology to the removal of a gland and its replacement by its hormone. The story of these decadal and multi-decadal rhythms in the past few years repeats the story of circadians, which, only half a century ago, were regarded as trivial associations of emotions, exercise and diet, until a genetic basis was clarified.

Non-overlapping 95% confidence intervals between environmental cycles and the near-matching biological cycles, the two associated by the "remove-and-replace" approaches just discussed suggest that some decadal or multidecadal rhythms are anchored in our gene pool. The intermodulations among cyclic mechanisms in the broader chronomes, in and around us, resolved by time series analysis, replace, as time-specified feedsidewards, the time-unqualified feedbacks or feedforwards (2).

Chronometaanalysis

Chronomics can provide much new information where the naked eye, even when accompanied by an analysis of variance, has limitations (62) or fails (63, 64; cf. 52). In an elegant study, thoroughly carried out, c-Fos immunoreactive cells in suprachiasmatic nuclei (SCN) of rats were studied around the clock at 2-hour intervals. The rats had been previously synchronized under two different lighting regimens, one group in light (L) for 16 hours and darkness (D) for 8 hours, i.e., LD16:8, the other on the opposite photofraction, in LD8:16. For the 24 hours of the experiment, the rats had been released into continuous darkness as a prolongation of the dark span. Each data series taken off the published graph was analyzed by a single cosinor at a trial period of 24 hours (notwithstanding an uncertainty that could not be clarified concerning one outlier). Parameter tests were applied for the purpose of two kinds of comparisons: first of any effect of the lighting regimens on the SCN as a whole and separately on each part of the suprachiasmatic nucleus, and second a comparison was made of the dorsomedial vs. ventrolateral part of the SCN on each of the two lighting regimens.

The chronobiological analyses, like the previously published results, found a statistically significant difference in c-Fos immunoreactive cells in whole SCN and in a separate dorsomedial and ventrolateral SCN parts between the two lighting regimens. In each case, the rats that had been kept in the long photofraction, LD16:8, had more c-Fos cells than the rats that had been kept under LD8:16. What the naked eye cannot quantify but can see, a comparison of the circadian amplitude shows no effect of the lighting regimen upon either the whole SCN or separately upon the dorsomedial or ventrolateral parts. Figure 15c shows the results of a comparison of the ventrolateral vs. dorsomedial SCN cells, separately on each of the two lighting regimens. The circadian amplitude is larger in the dorsomedial than in the ventrolateral SCN, whether the rats were kept in LD16:8 or LD8:16. There was, however, a statistically significant difference in the MESOR of c-Fos immunoreactive cells, depending on the site in the SCN, the ventrolateral location being associated with a smaller number of c-Fos cells than the dorsomedial site (not shown). In order to account for this difference in MESOR, the amplitudes were expressed as a percentage of the MESOR, and the comparison of ventrolateral vs. dorsomedial cells at each lighting regimen was repeated. As seen in Figure 15b as % of mean, the circadian amplitude differed between the dorsomedial and ventrolateral parts only for rats that had been kept in LD16:8 and not for rats that were kept on the regimen with a short photofraction, LD8:16,

as also shown in Figure 15c, which displays the fitted cosine curves as a function of time to the data expressed as a percentage of the series mean. There is thus an effect of one of the lighting regimens on the circadian amplitude of the c-Fos immunoreactive cells, even after the difference in MESOR has been taken into account, showing an effect in rats exposed to the long but not in those on the short photofraction.

The latter results, irrespective of timing, can be regarded as particularly pertinent in any comparison of biological chronomes exposed to more vs. less light per day. This problem was considered with focus on multiseptan rather than circadian rhythms, for comparisons of dental chronomes in contemporary vs. ancient civilizations, the latter without artificial light (65). From a methodological viewpoint, Figures 15a-c show the quantitative inferences that can be drawn by chronomics that provide a more powerful approach than an analysis of variance (66). The merits of discussing separately the different characteristics of rhythms, adds in the case of Figure 15 the consideration of the amplitude to that of an average (for the sake of brevity the phase difference also apparent when the zero phase became "light on" is here not considered). Figure 15 is intended to show that the scope of time-macroscopically valuable research can be extended by quantification via chronomics. In another case cited, chronomics have detected and quantified a melatonin rhythm objectively (52). There, the naked eye not only failed to quantify multiple components (52) but missed the occurrence of a rhythm as such (63, 64). In each case, major macroscopic opinion leaders were involved.

Problems for the future: 7-day/24-hour monitoring of newborns' blood pressure dynamics

Based on the maximum sampling allowed by the "ethics" of the time, blood pressure and heart rate could be autonomically measured half-hourly on healthy human newborns only for 48 hours. Such data in 1985-86, summarized by cosinor as a group phenomenon, show a circadian rhythm of systolic blood pressure in the case of a positive (top) but not in that of a negative (bottom) family history (FH) of high blood pressure and/or other vascular disease, Figure 16a. During the years 1987-89, the circadian amplitude of blood pressure in newborns had increased with statistical significance in babies with a negative, but not in babies with a positive FH, Figure 16b. We sought but could not find any difference in the way the babies were handled, except that the years 1985-86 coincided with a solar minimum, and the ensuing years with the ascending stage of solar activity in the ~10.5-year sunspot cycle. Clearly, we needed data over spans longer than 48 hours to understand circulatory dynamics in the first week of life, a task to be accomplished in keeping with the then still-prevailing "ethic" that healthy newborns are not to be monitored for more than 48 hours at half-hour intervals. The solution came from the circumstance that some babies could be studied for 48 hours during the first two days, while others could be studied during some other two consecutive days in the course of the first post-natal week. Thereby, we could explore, with serially independent sampling, any weekly component in the data pooled (from dozens of babies, eventually from a total of 164 babies each providing 48-hour series). From the integration of such separate 48-hour observations a weekly pattern could be constructed, Figure 16c, shown to differ as a function of family history, in the time domain, Figure 16d, and in a polar cosinor display in Figure 16e.

Longitudinal sampling on the same individual in health for the first 40 days of life has confirmed the initial great prominence of the biological week (67). The systematic individualized sampling of groups of babies in health is indicated preferably for longer than the first week of life, to seek a test in the newborn that should assess the risk of developing a high blood pressure later in life, irrespective of solar cycle stage. In considering these results that should prompt further work, we undertook, during the past decade, the hazardous journey from the newborn's putative sensitivity to the solar cycle stage to broader effects of the cosmos, that appear perhaps in the form of magnetolability and lead to associations of morbidity and mortality and many human affairs with various aspects of our physical environment, Figures 8-13.

For practical vascular disease risk assessment at all ages, we require long-term, at least week-long monitoring (until lifetime surveillance becomes practical), as the basis of a preventive health care that acts before the *fait accompli* of disease, that detects changes in the usual value range and responds to the changes in dynamics therein. On the positive side, this is documented for adults based on a summary of 2,736 individuals monitored by 158,177 measurements. The steps taken by Jarmila Siegelova, in well over 100 published titles within a decade, starting with the timing of aspirin (68) and most recently dealing with circadecadal cycles (69), originally with Pavel Prikryl as well as with Bohumil Fiser, Jiri Dusek and Pavel Homolka, and even earlier in the footsteps of Mendel's concern for meteorology all led to a cooperation between Brno and Minnesota. Our original approach to the prevention of vascular disease that worked on a population basis at different ages for 2 years was overpowered by the solar cycle at the very sensitive pre- and perhaps perinatal age that may be characterized by magnetolability under circumstances yet to be elucidated. We are trying to make a confounder into a friend by pertinent methodology (70). Whether these chronomes will also elucidate intentionality (71) and the dimensions of physicists approaching emotions, the mind and spirituality in purely physicomathematical terms remains to be seen. This 82-year-old can tell about this beginning, but others will live to complete it. From a physical viewpoint, the prominent Stanford professor emeritus William Tiller writes (71):

> The goal of science is to gain a reliable description of our natural phenomena, so as to allow accurate prediction within appropriate limits, of nature's behavior as a function of an ever-changing environment ...

Indeed we need

> internally self-consistent knowledge about the relationships between different phenomena and different things.

(For "different things" read "cycles".) In these definitions, with which we agree, useful but as yet missing information relates to the time horizon, the information on the very many rhythms as they organize chaos and undergo trends, i.e., the information on the chronomes of an ever-changing demography. There is a need for mapping by chronomics the entities involved in these cycles, indeed the sets of phenomena that share the wave particle duality (71). Against this background, the mapping of quasi-spontaneous cycles in religious activity and the mapping of other cycles related to the brain, some perhaps antithetical ones such as those involved in criminality and war, may be the challenge of those interested in both the ills of the individual that seem to be much more amenable to an empirical approach and

the ills of society that are much in the forefront since the terrorist attacks of September 11, 2001, and require an even more urgent solution.

Glossary

The introduction of terms here has been guided as previously (72, 73) by the consideration of brevity, but also of familiarity with at least part of a neologism (the ringing of a bell) and most importantly by staying away from implying any mechanism involved. Instead, the basis of the terms is the numerical indication of the length of the period, in preference usually to the (reciprocal) frequency, since a majority of people can think easier in the time than in the frequency domain, notably in the case of long periods. Exceptions such as "ultradian" and "infradian", relating to frequencies rather than to periods, are prompted by the desire for using the precedents of "ultraviolet" or "ultrasound" for frequencies higher than the visible or audible, and likewise we have the precedents of "infrared" and "infrasound" for frequencies lower than the visible or audible. Many terms will require compromise to reach a unified transdisciplinary terminology in view of the discrepancies apparent in Table 4 between the frequencies introduced by physicists and engineers and those of biologists on the other. For biomedicine, the very low frequencies of the physicists are relatively very high. The terms here prepared or their equivalents in English will have to replace the "high", "low", "very low" and "ultralow" frequencies currently used by scholars in heart rate variability, as HF, LF, VLF and ULF. It is not only clear that these frequencies are far from being the lowest, when heart rate variability is shown to exhibit a circadecadal rhythm. Eventually, the currently broadened system time for an ECG from a few minutes to 24 hours or to a few weeks, but as yet only on demand, will be switched to continuous surveillance with as-one-goes windowing, compacting and broader and broader recycling by repeated passes over the larger and larger accumulating data set by broader and broader moving cosinor windows.

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Figure 1. Emergenic heritability of the circadian amplitude of the human heart rate.

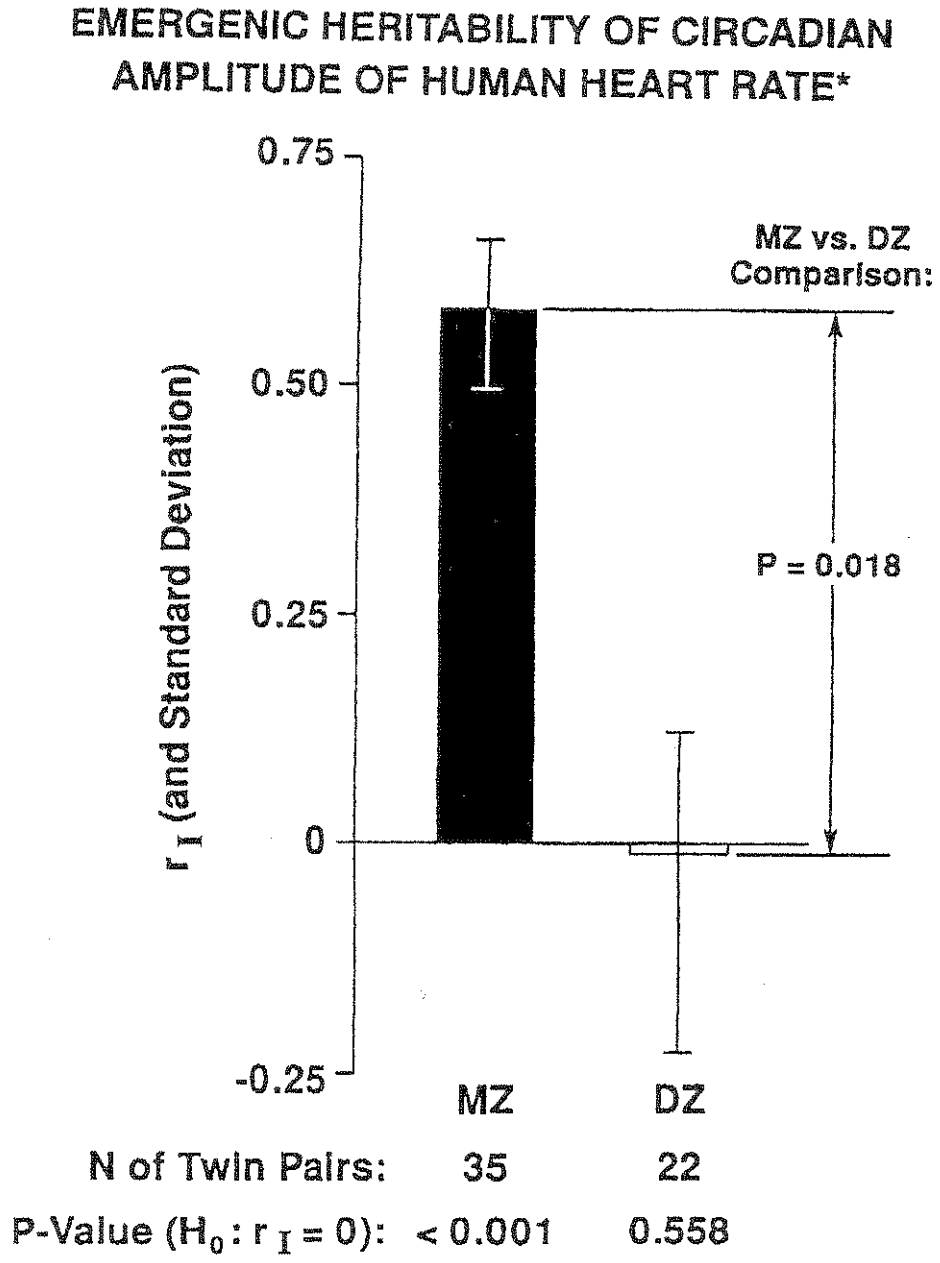
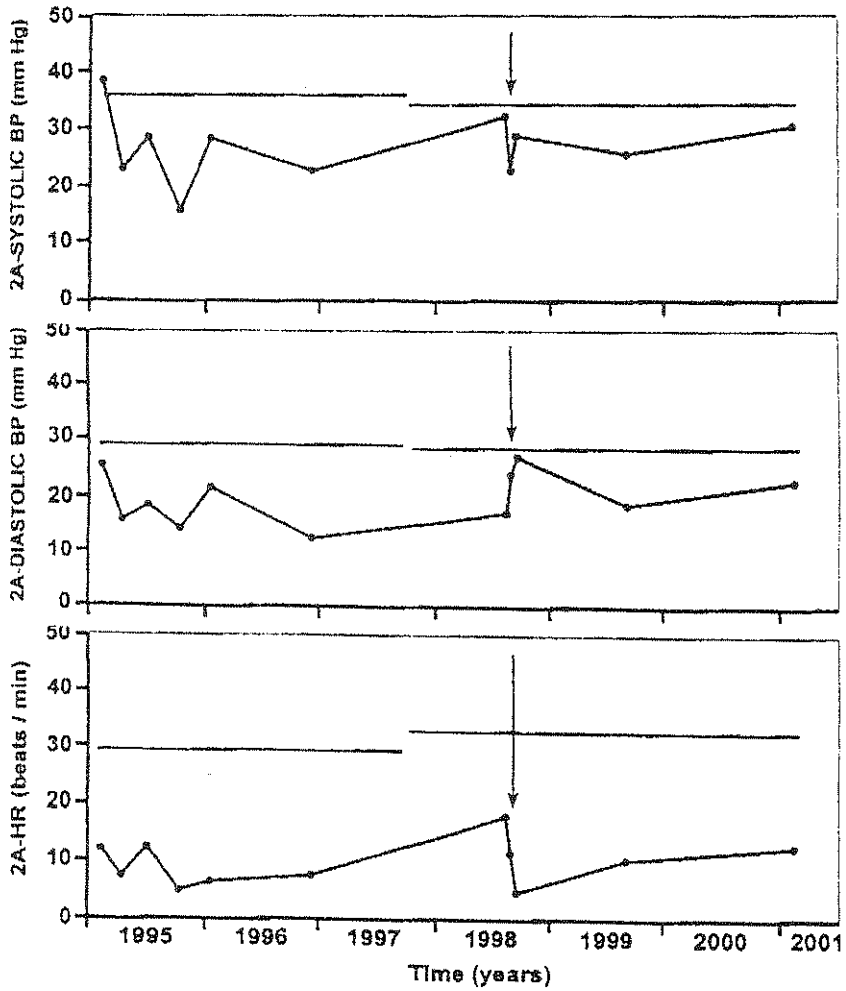


Figure 2. Less than 8 or 80% morbidity in the absence (left) and presence of two separate disease risks (middle) that can occur in the otherwise neglected range of physiological variation, a putative dividend from seeking a more reliable diagnosis.

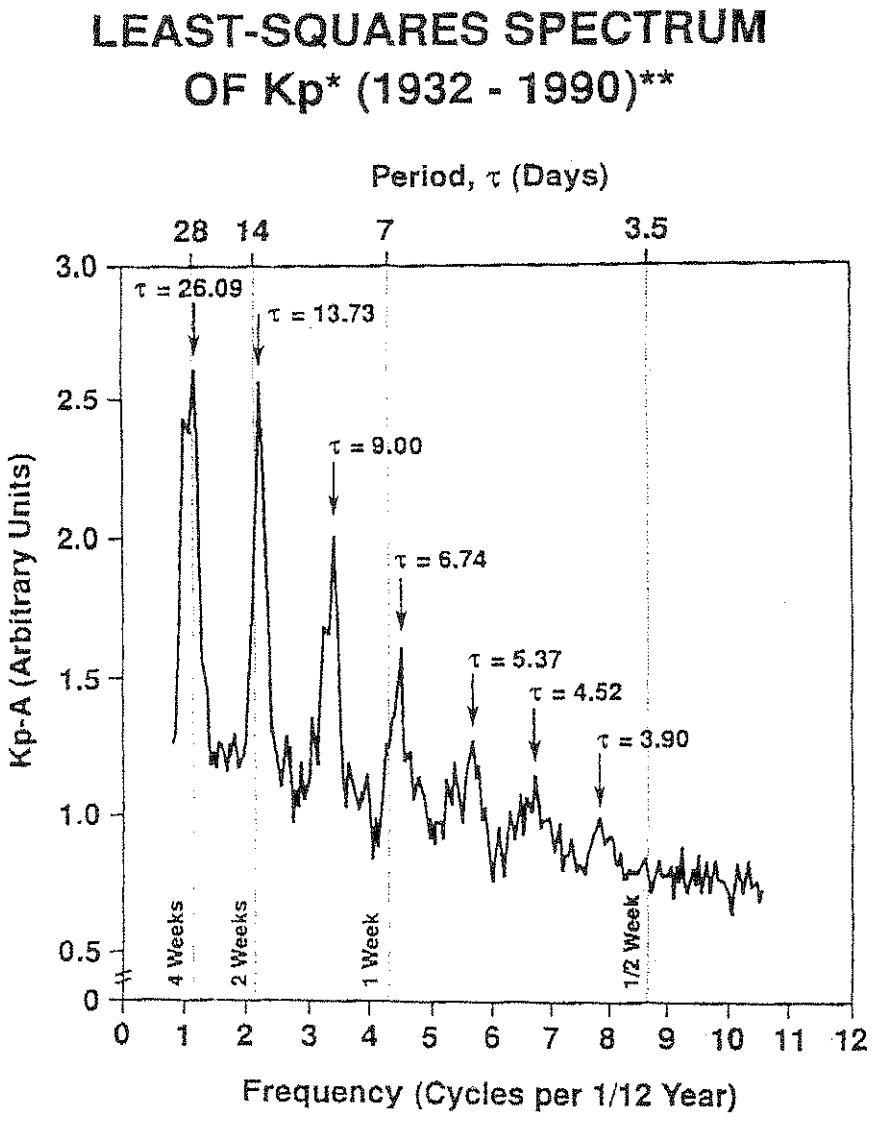
Figure 3 (CH). The circadian amplitude, in this case of transient "24-hour" systolic circadian hyper-amplitude-tension (24-hour CHAT), shows an overall elevation in a first over 20-day around-the-clock profile, but not during the ensuing 6 years.

SIX-YEAR COURSE of CIRCADIAN DOUBLE AMPLITUDE (2A)* of MATERNAL BLOOD PRESSURE (BP) and HEART RATE (HR)



* CH, 60 years of age at start. Each dot represents a profile of measurements at 30-minute intervals for at least a week, usually for a longer span (N = 340 to 855 / profile, total: 6115). Arrow: learning about diagnosis of ovarian cancer. In no case is sequential test (by CUSUM) or the fit of a linear trend statistically significant. Except for first dot there is no further "profile-CHAT", occasional "~24-h-CHAT", shown elsewhere, notwithstanding. The findings of "~24-h-CHAT" with or without "profile-CHAT" are another reason for preparing about 24-hour in addition to at least week long (profile) summaries. Horizontal lines: upper 95% prediction limit adjusted for gender in different age categories.

Figure 4. Least-squares spectrum of the geomagnetic index (Kp) recorded every 3 hours for 59 years between 1932 and 1990, obtained by a population-mean cosinor spectrum, summarizing results over consecutive years. Large-amplitude peaks at periods of about but not exactly 28 days, 14 days and 7 days can readily be seen. Although the about 28-day component has long been associated with the relative motion of the moon around the earth, when it comes to geomagnetism, the about 27-day rotation of the sun around its axis has to be considered as a major source of variability in the circatrigintan range. The main point of this figure, however, is a component of 6.74 and not precisely 7 days (23). It was also subsequently found in aa (24) and in the record of a stand-alone magnetometer 600 km from the nearest human habitation in Antarctica (25).



* Index of disturbance of the horizontal component of the geomagnetic field

** Least-squares fit of harmonics, separately to 3-hourly values of each calendar year, using steps equal in frequency between 10 cycles/year and one cycle per 70 hours; A = average amplitude

Figure 5. Large circasemiannual component with an amplitude much larger than the yearly component is apparent in phase-unweighted (left) and phase-weighted (right) spectra of Kp along with the near but not exactly weekly components.

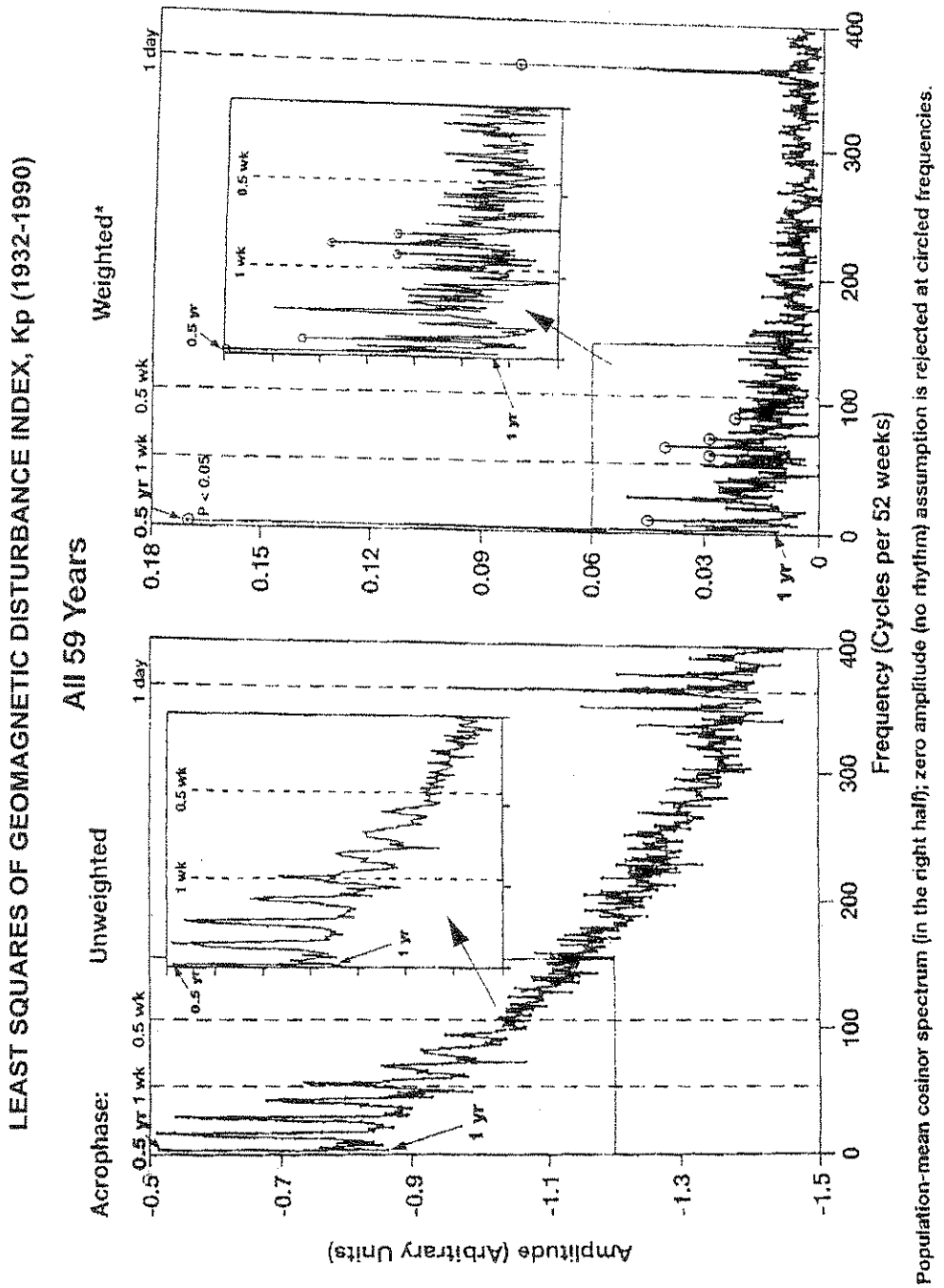


Figure 6. The naked eye can see an about 10.5-year cycle relatively clearly, as well as modulations with lower frequency that, however, for quantification require chronomics in the frequency domain, e.g., in Figures 7.

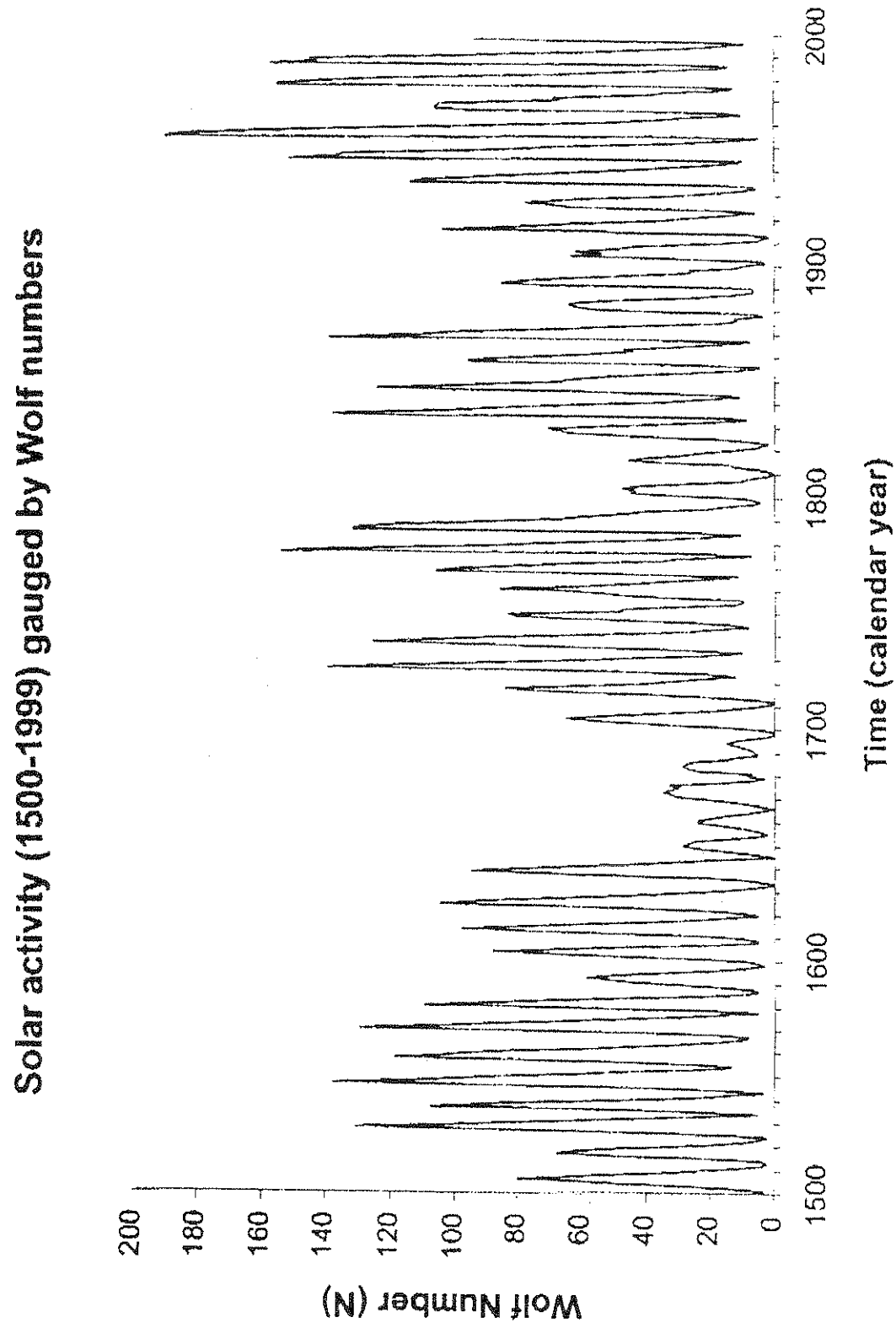


Figure 7. Multiple components are seen in the spectrum around 10.5 years on the right and other components with a much lower frequency on the left.

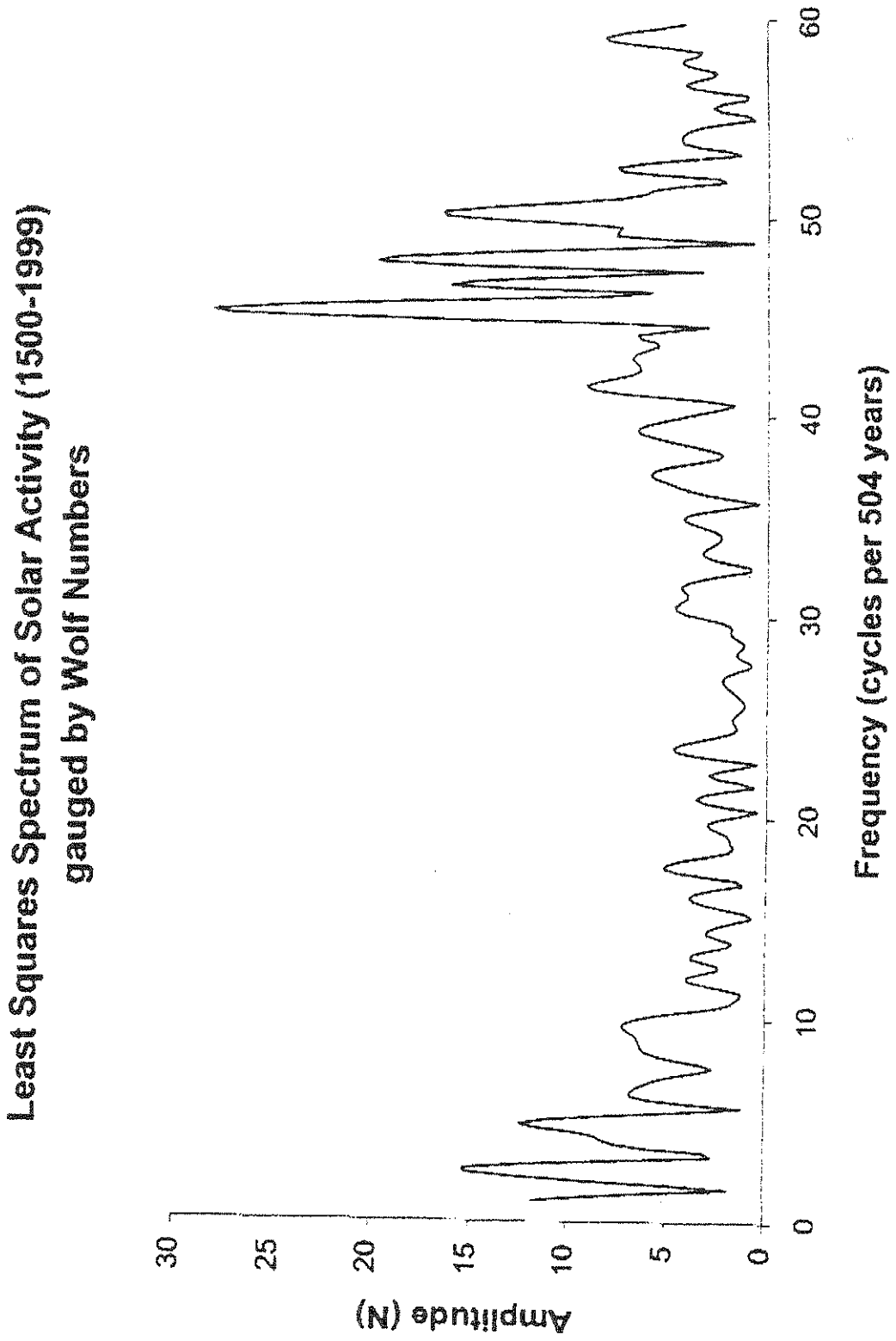


Figure 8. Broad scope of unselected about 10.5- and about 21-year cycles. Note that both components can be represented in the same variable, such as natality or murder.

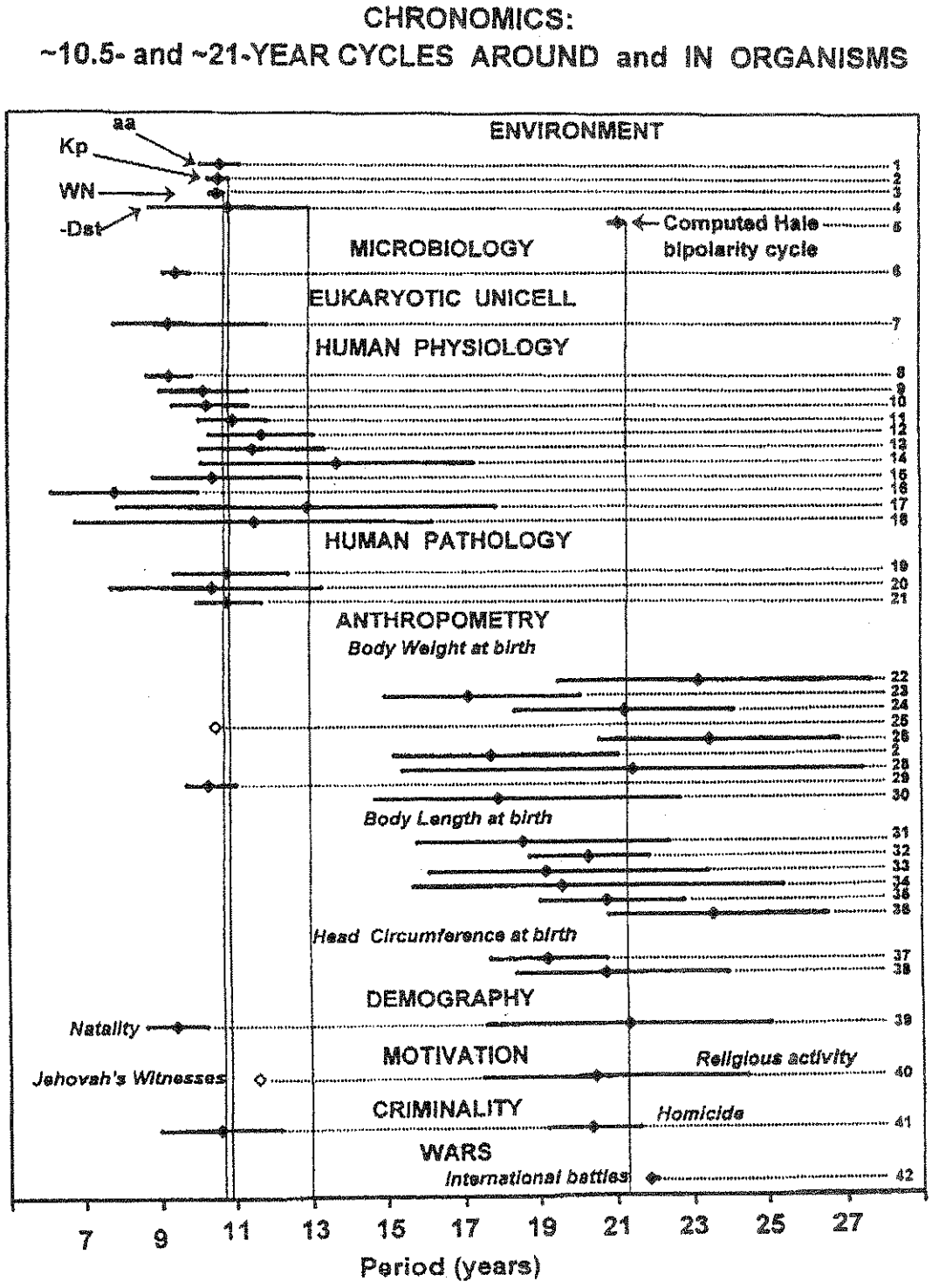


Figure 9. Time relations of 21-year near-matches of the Hale cycle on the earth's surface. Non-overlapping 95% confidence intervals provide a width of horizontal bars, suggest the operation of different mechanisms in bringing about phase differences in the same variable, even in the same location for different ethnic groups.

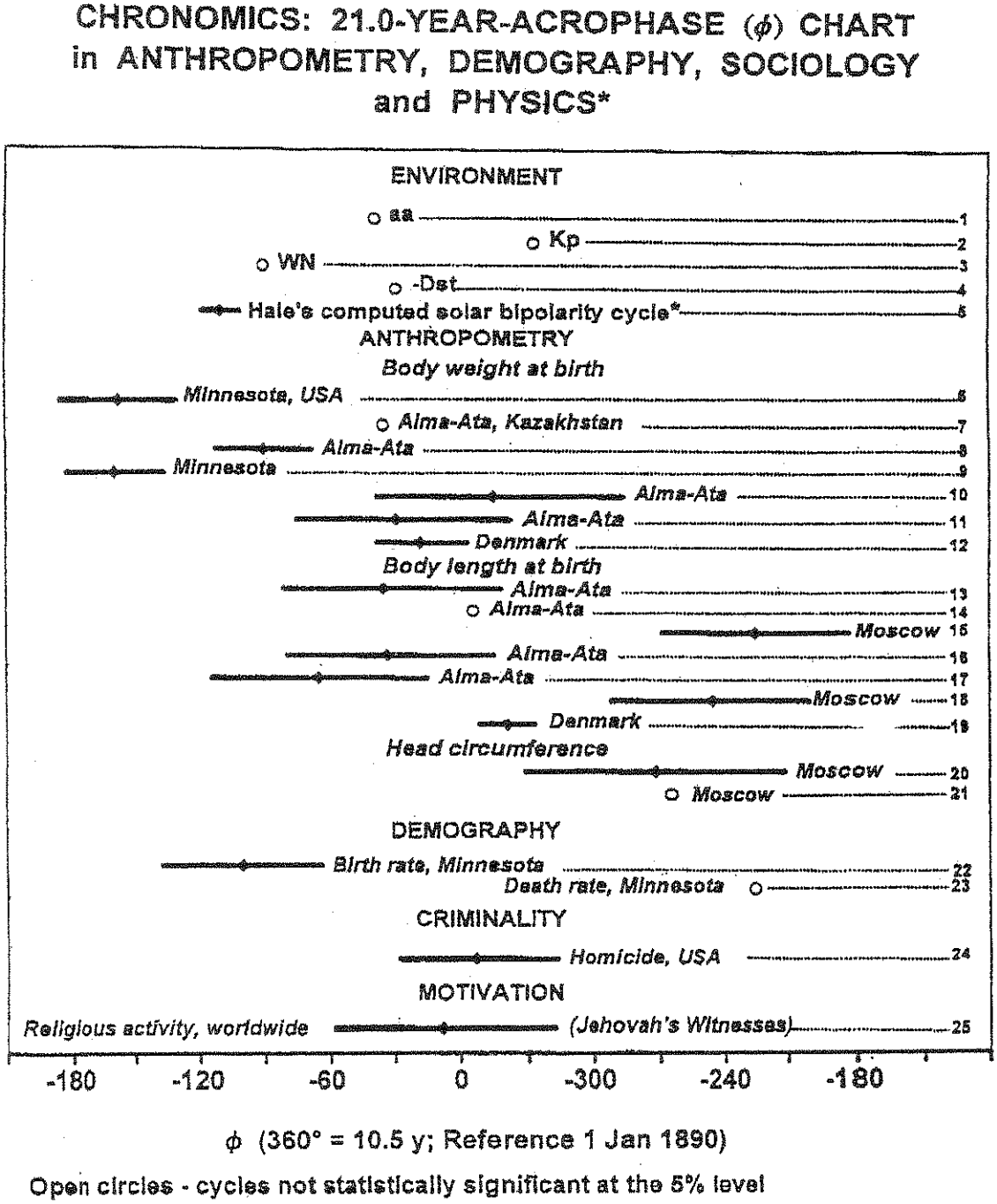
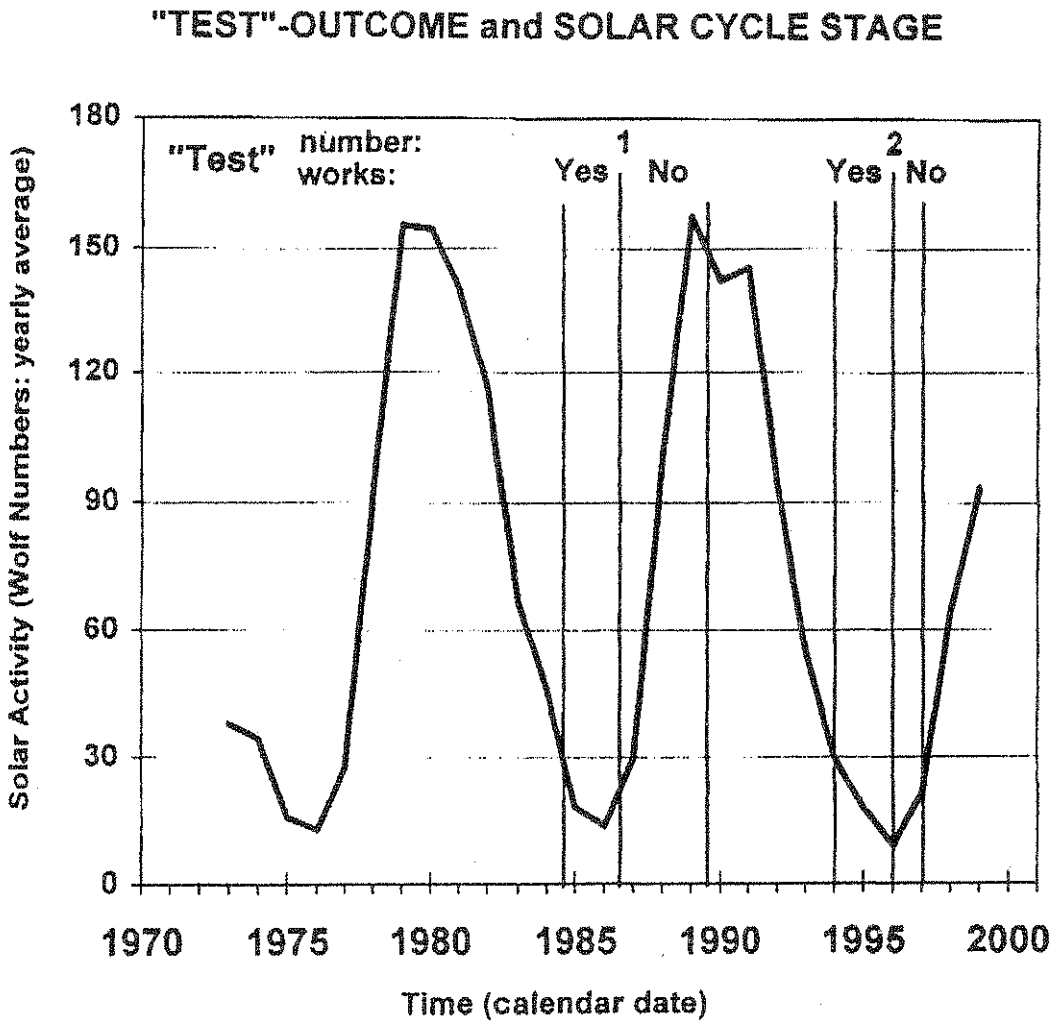
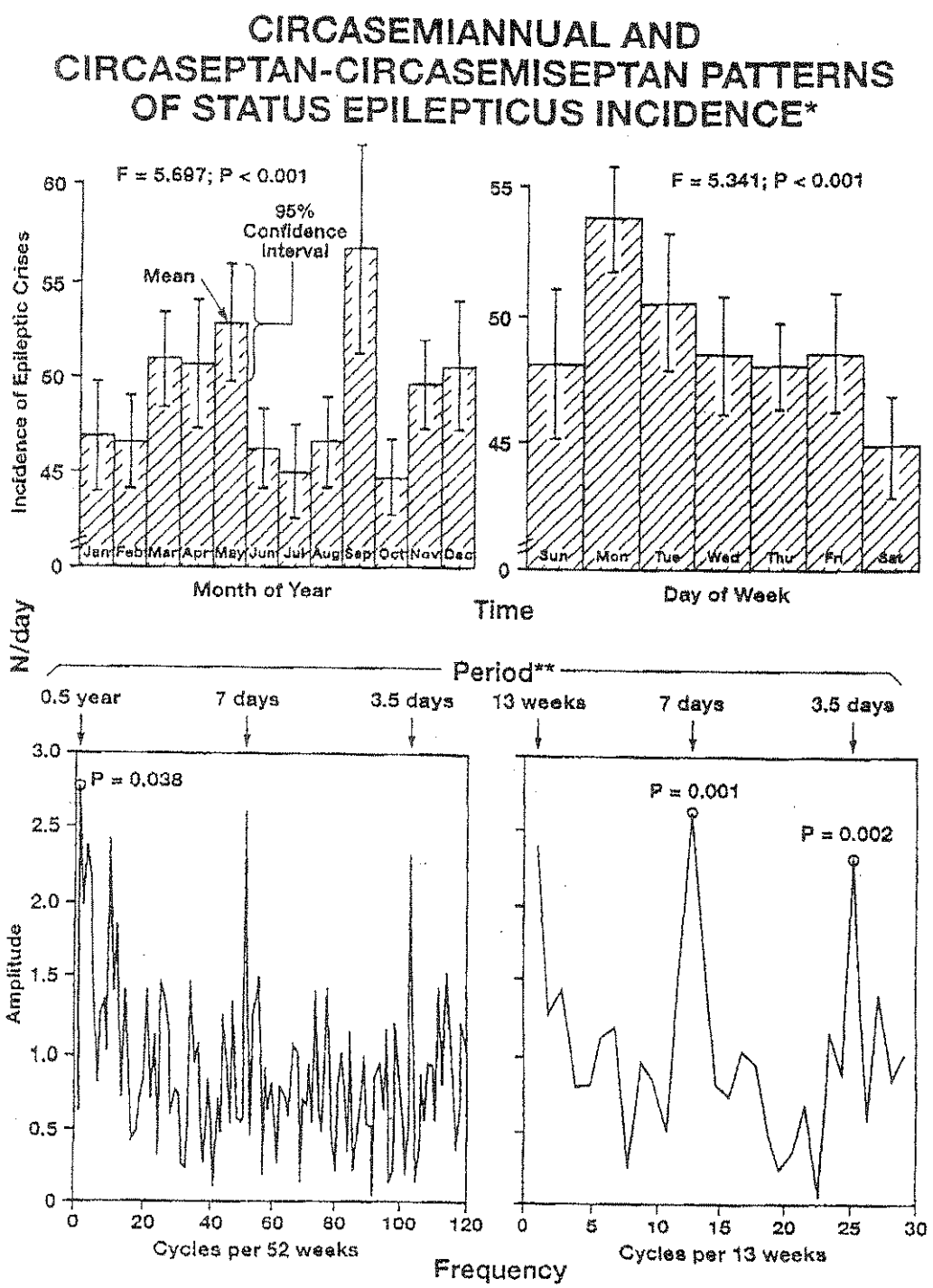


Figure 10. The solar cycle stage provides a time horizon in which different results obtained by sampling along much shorter system times such as a day or two (test 1), or of a few years (test 2) may be further tested when they differ with solar cycle stage. This is the case for a test of circadian amplitudes of blood pressure for distinguishing newborns with a positive vs. negative family history of high blood pressure or other vascular disease (test 1). Cf. Figure 16a and b. Solar cycle stage dependence may also apply (test 2) to the presence vs. absence of a circasemiannual modulation of nightly melatonin at middle latitudes at solar minimum vs. the ascending stage of solar activity (37 and unpublished).



Test-criterion, for groups only:
1 - higher circadian amplitude of blood pressure in babies with a positive versus negative family history of high blood pressure in 48-hour measurement series at 0.5-h intervals; 2 - nocturnally circulating melatonin undergoes circasemiannual variation.

Figure 11. Display in the time domain (top) and the frequency domain (bottom) of about half-yearly (left) and about-weekly (right) patterns in status epilepticus. Please note in the lower display on the left that the half-yearly spectral component is much more prominent than the yearly one.



* From 53,616 cases recorded in Moscow, Russia between 1 Jan 1979 and 31 Dec 1981.

** P-values from population-mean cosinor on 3 yearly estimates (left) or 12 3-monthly estimates (right). Overall least squares spectrum finds 0.5-year, 1.0- and 0.5-week components statistically significant ($P < 0.001$) (in addition to about 33.1- and 28.0-day components).

Figure 12. Illustration of the about 21.0-year component in the average number of hours spent working for their church per month by Jehovah's Witnesses for the grand total, including other locations in addition to the 103 sites analyzed separately. Model fitted by nonlinear least squares consists of linear trend and cosine curve with trial period of 21.0 years. Whereas a linear trend applies to the grand total, such a trend is statistically significant in only about 50% of the individual data series, and can be negative as well as positive.

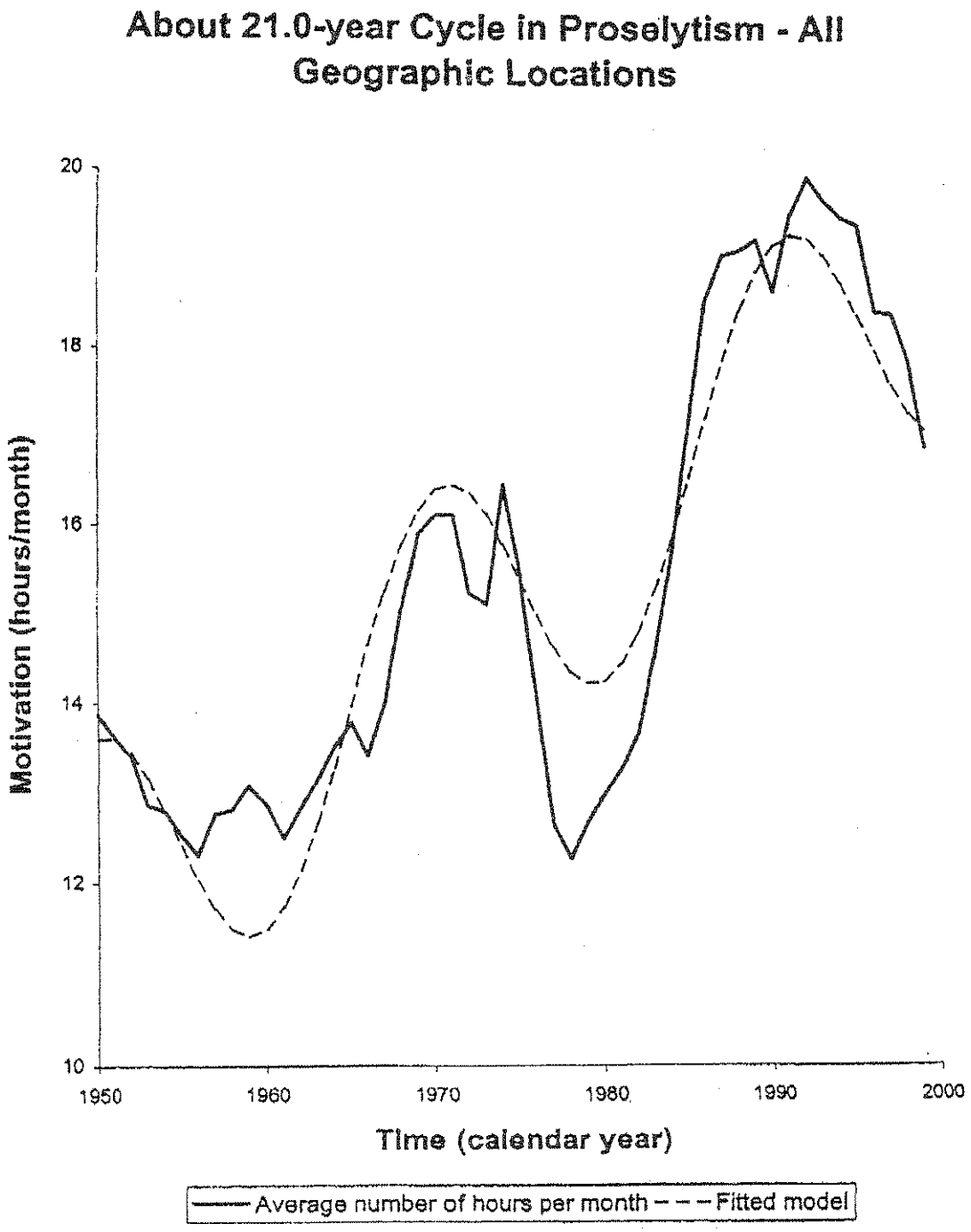


Figure 13a. A prominent about 50-year component is apparent to the naked eye, with the analysis revealing a second about 21-year component as well, the composite shown by a dashed fitted curve.

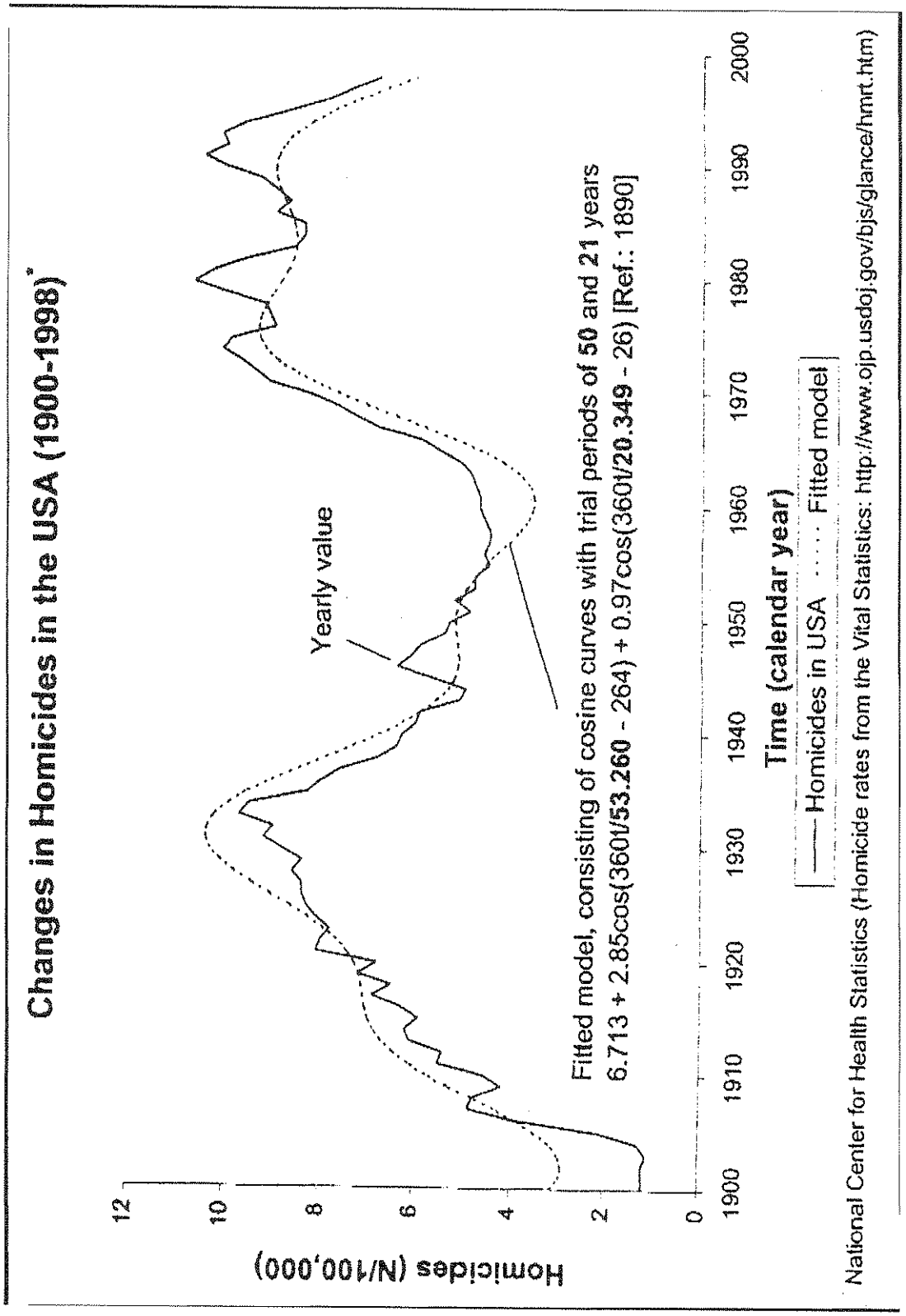


Figure 13b. Diversity of about 50-year cycles for comparison with other multidecadal rhythms in Figure 8.

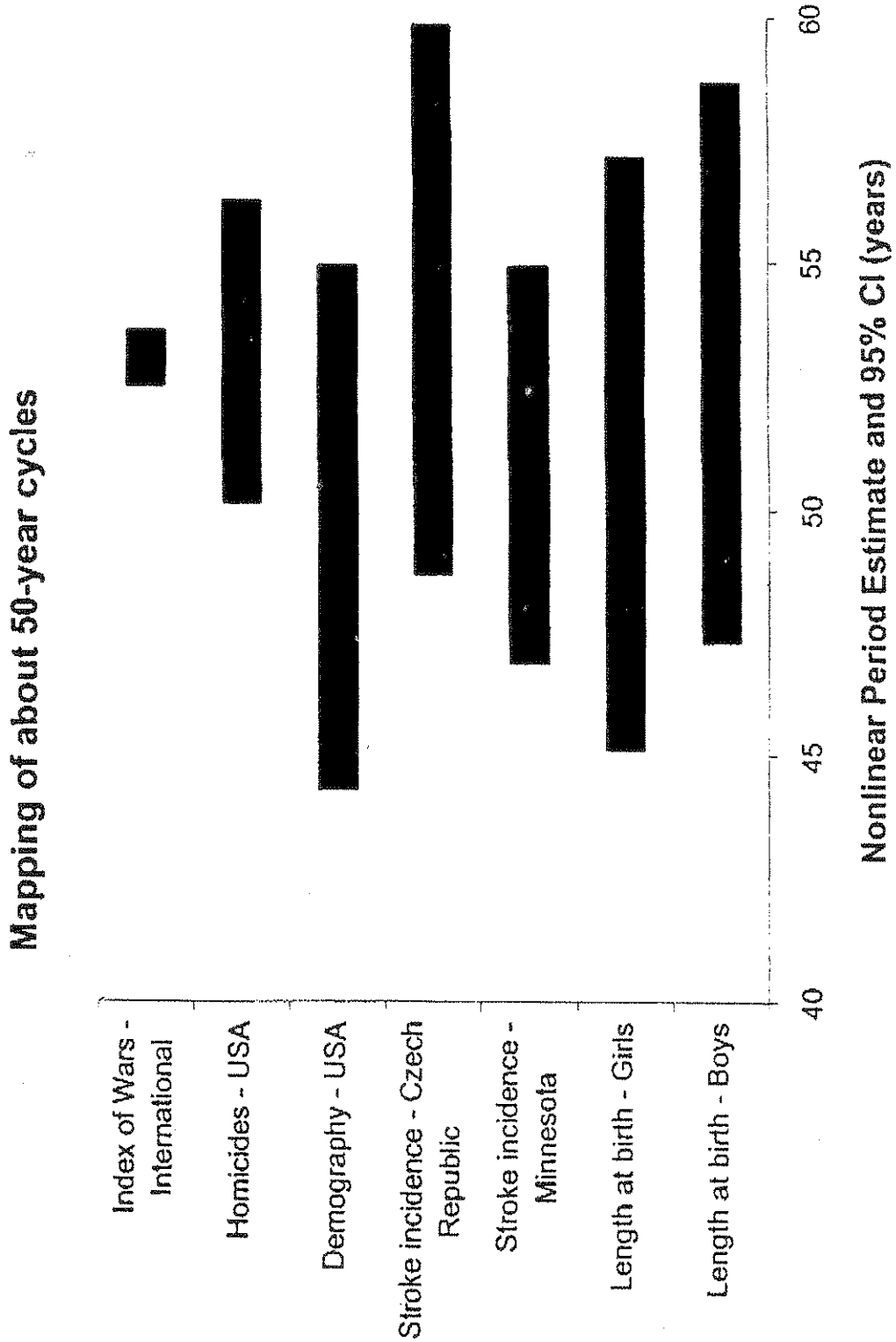
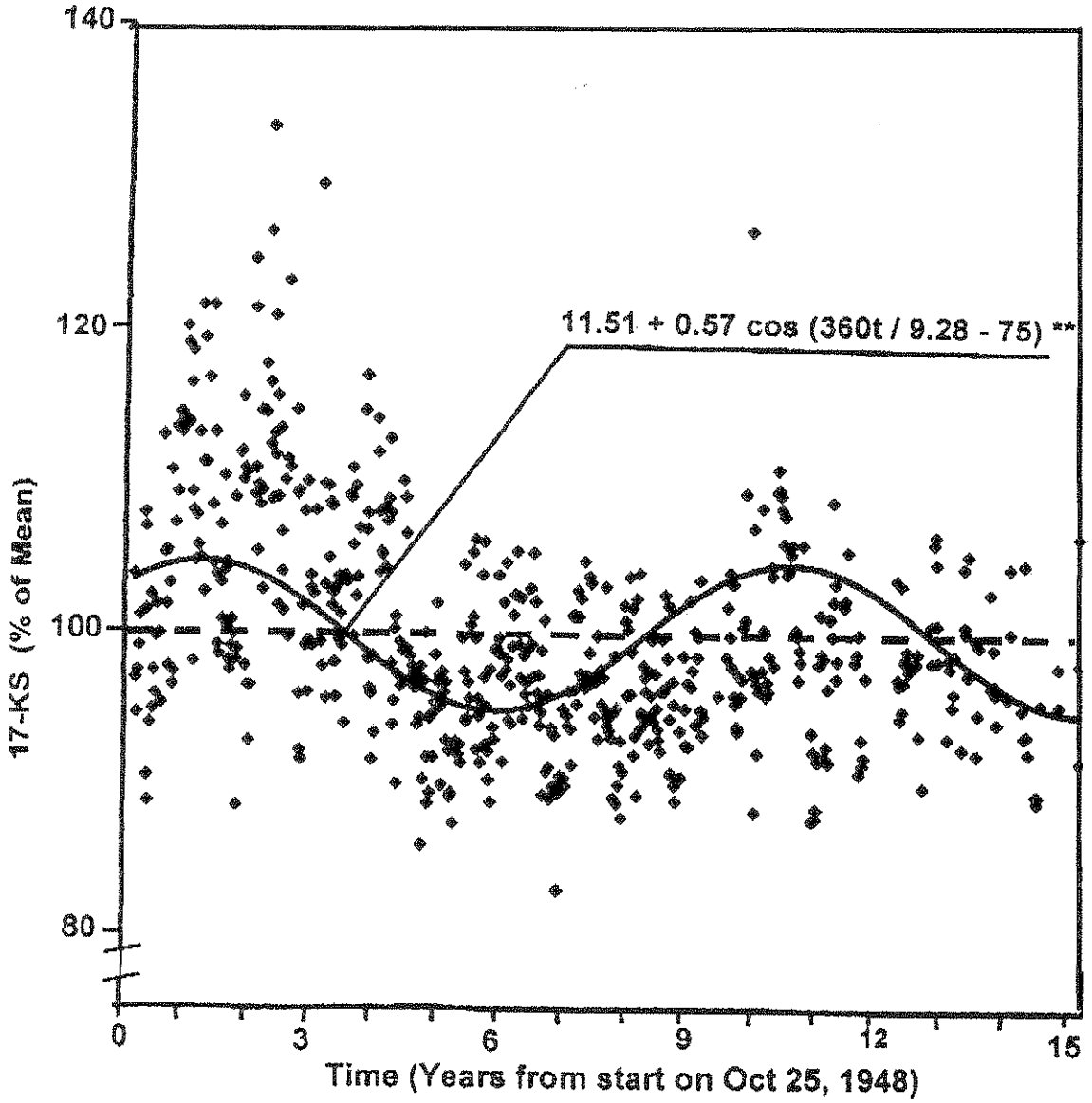


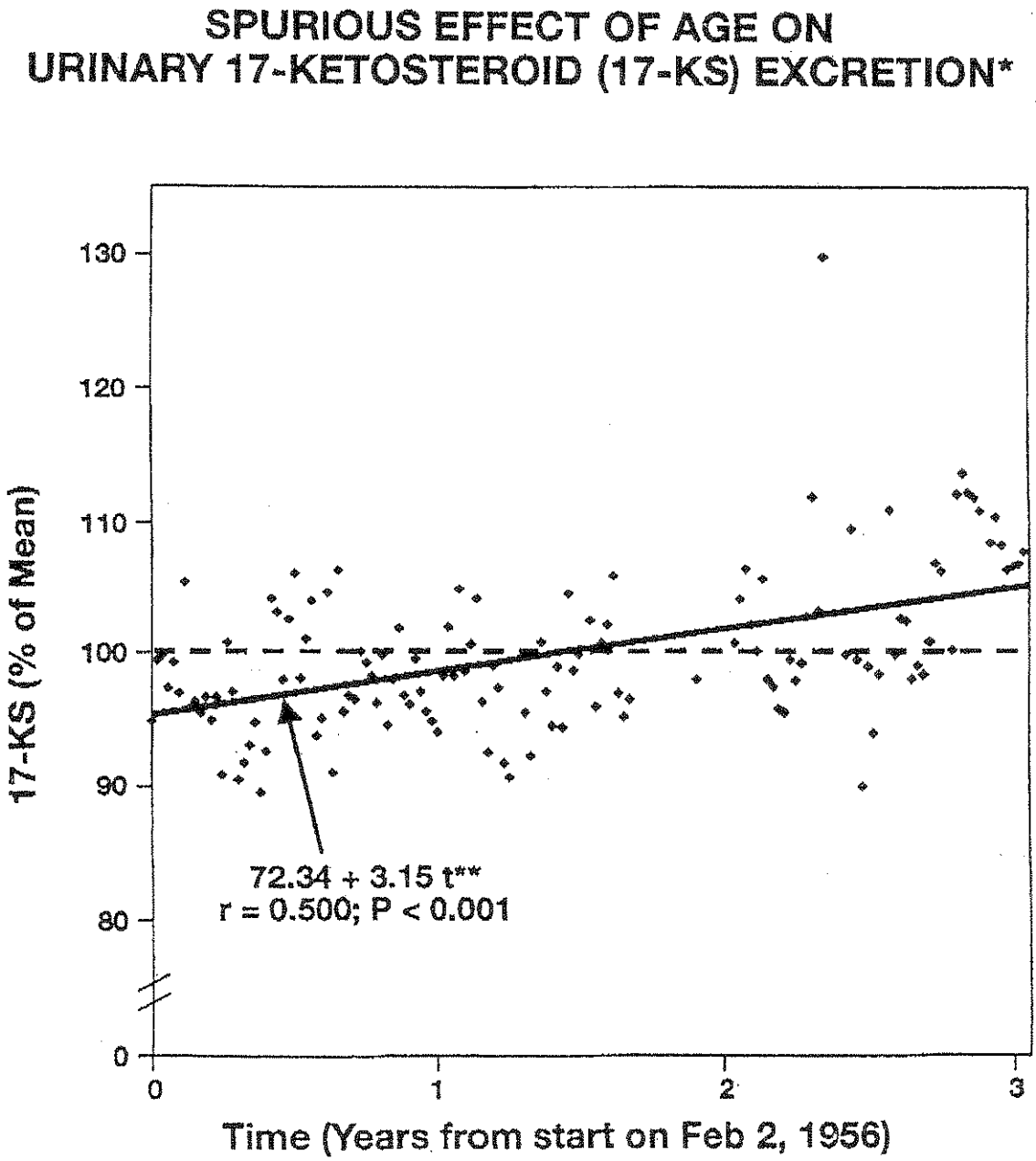
Figure 14a. Had this figure been submitted as such to any leading journal publishing case reports, the high statistical significance of the change with age with maturity may well have led to acceptance.



* By clinically healthy man (CH, 43 - 58 y); weekly averages.

** Model validated nonlinearly with period of 9.28 years, with a 95% confidence interval (CI) extending from 8.72 - 9.95 years, non-overlapping the CIs of nearest periods in geomagnetic disturbance (Kp) or in solar activity (Wolf number).

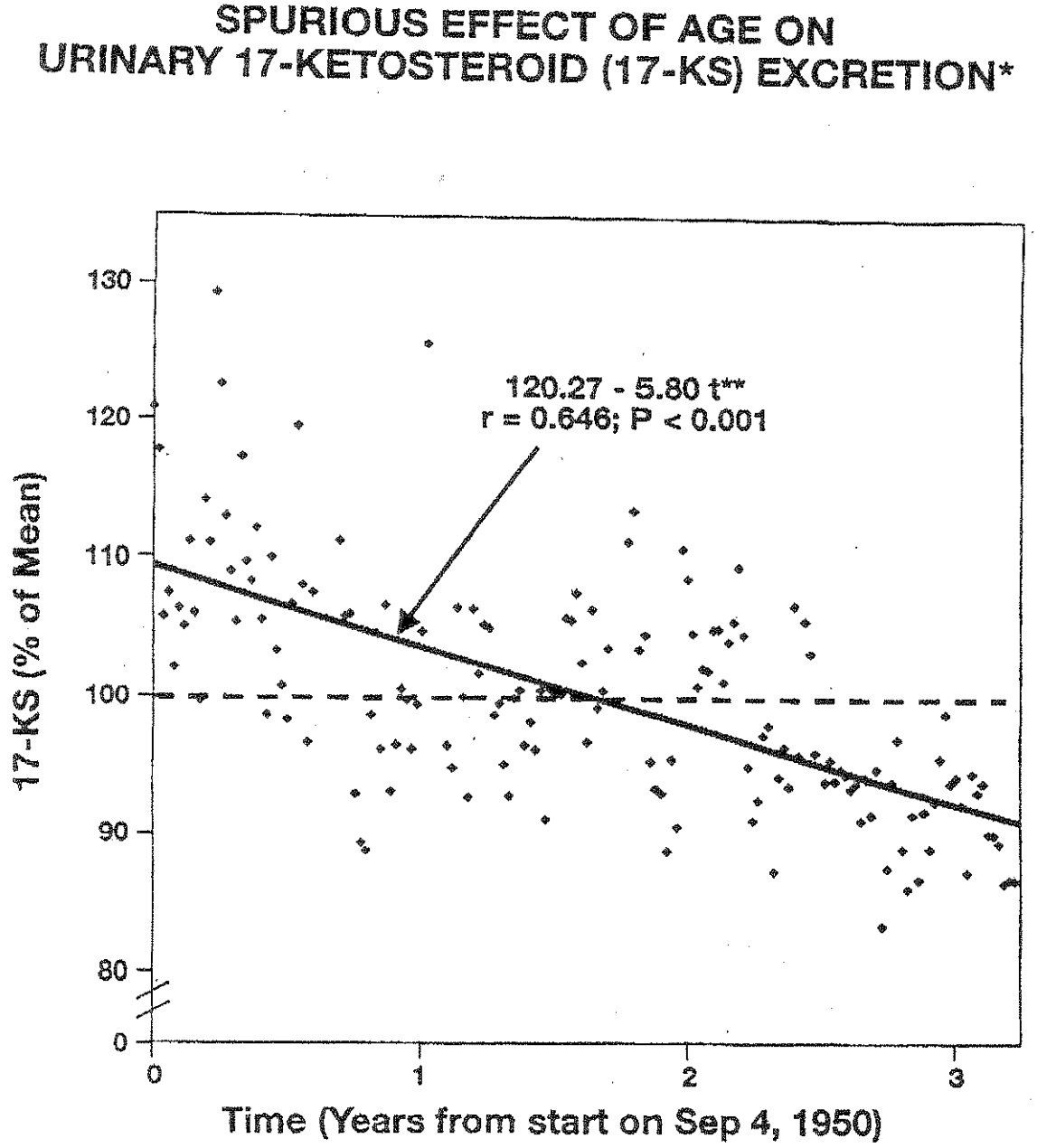
Figure 14b. If submitted separately, an effect opposite to that in Figure 17a may perhaps also have been published as "paradoxical".



* By clinically healthy man (CH, 52-54 y); weekly averages undergoing ~9.3-year cycle.

** t in years (from 21 Dec 1947).

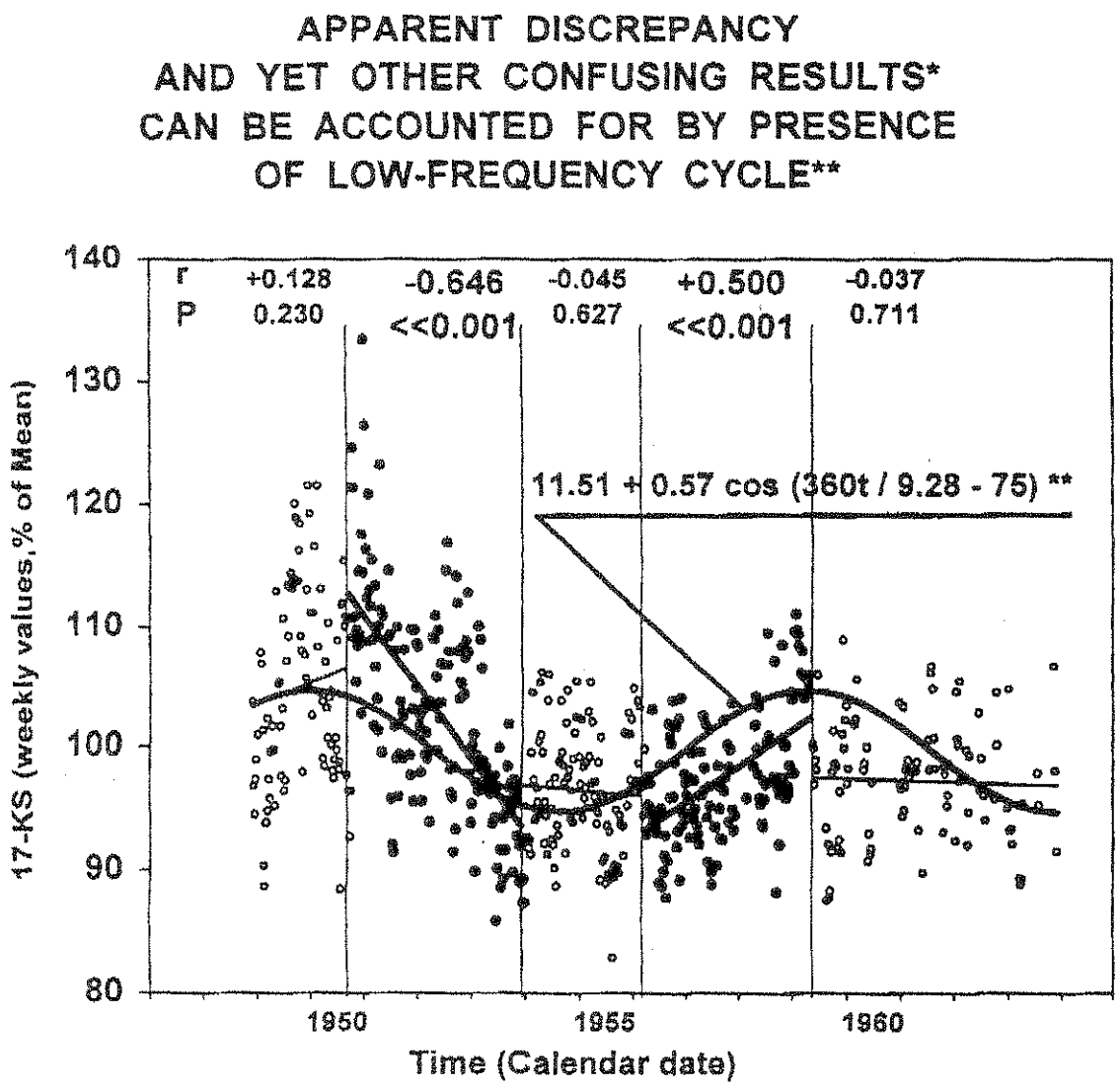
Figure 14c. The foregoing two figures (12 and 13) may be accounted for by a rhythm.



* By clinically healthy man (CH, 46-49 y); weekly averages undergoing ~9.3-year cycle.

** t in years (from 21 Dec 1947).

Figure 14d. When isolated parts of the entire record in Figure 14c are analyzed in addition to the two nonsense correlations in Figures 14a and b, no correlation may be found in the first, third and fifth section of this same record: more confusion?



* See five different regression lines with age.

** Model validated nonlinearly with period of 9.28 years, with a 95% confidence interval (CI) extending from 8.72 to 9.95 years, ref 21 Dec 1947.

Figure 14e. This figure reveals geomagnetic Kp and solar activity cycles, WN, during the span investigated on the subject studied in Figures 14a-d.

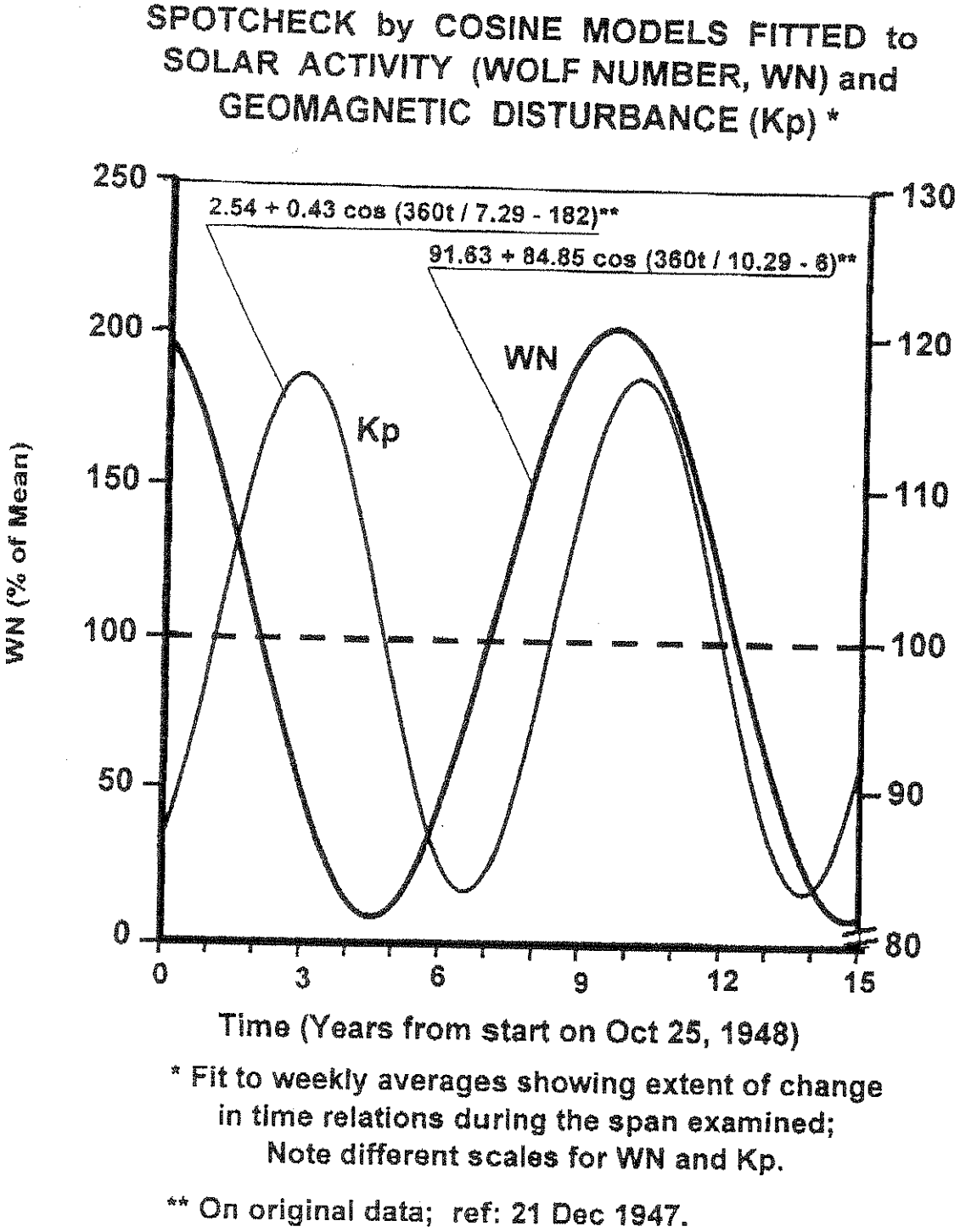


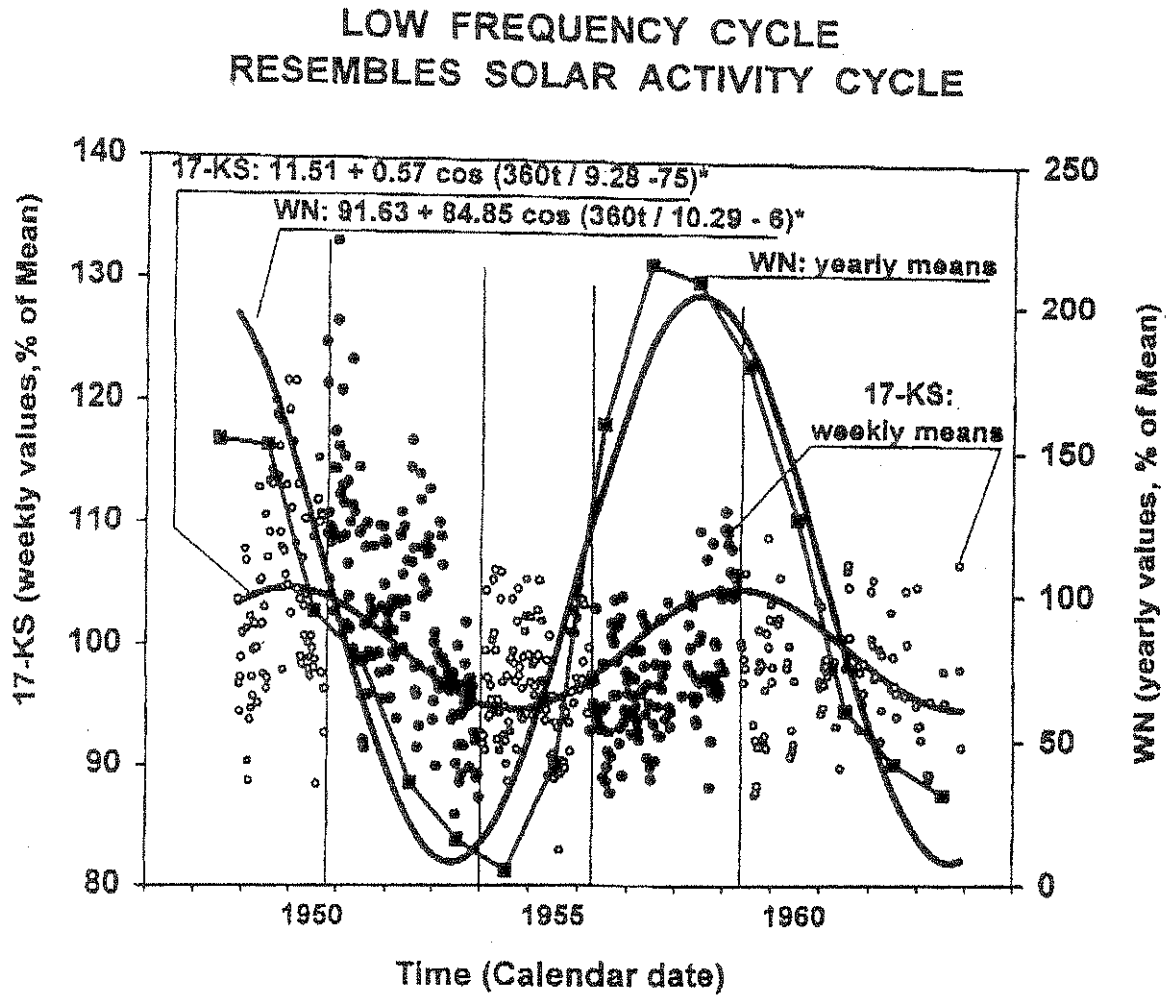
Figure 14f. Correlations of the urinary excretion of 17-ketosteroids with solar activity can display a high statistical significance, as in the second section of the display on the left, or no significance whatsoever, as in the rest of the display on the left, notwithstanding a cross-spectral coherence and the proper time relations with a maximum 17-ketosteroid following that in solar activity, both in keeping with a possible association.

WN = Wolf numbers, gauging solar activity; 17-KS = urinary excretion of steroidal hormonal metabolites (17-ketosteroids); an association and mediation by geomagnetic activity (Kp) is supported by a cross-spectral coherence of 0.588 between 17-KS and Kp at 1 cycle in 4.3 weeks (away from spectral peaks).

* Both as a function of time (age; not shown) and between the two variables (r: correlation coefficient; P: P-value from test of H_0: r = 0)

** Model validated nonlinearly, reference 21 Dec 1947.

Figure 14g. Data underlying the cross-spectral coherence between an endocrine mechanism of resistance to infection and the solar activity cycle associated with cholera epidemics by Chizhevsky in 1920.



WN = Wolf numbers, gauging solar activity; 17-KS = urinary excretion of steroidal hormonal metabolites (17-ketosteroids); an association and mediation by geomagnetic activity (Kp) is supported by a cross-spectral coherence of 0.588 between 17-KS and Kp at 1 cycle in 4.3 weeks (away from spectral peaks).

*** Model validated nonlinearly, reference 21 Dec 1947.**

Figure 14h. A partial steroidal chronome map in which all the periods isolated thus far with statistical significance are indicated with their relative prominence (amplitude). Note that a very prominent circadian (arrow) is modulated by a sizeable decadal spectral component.

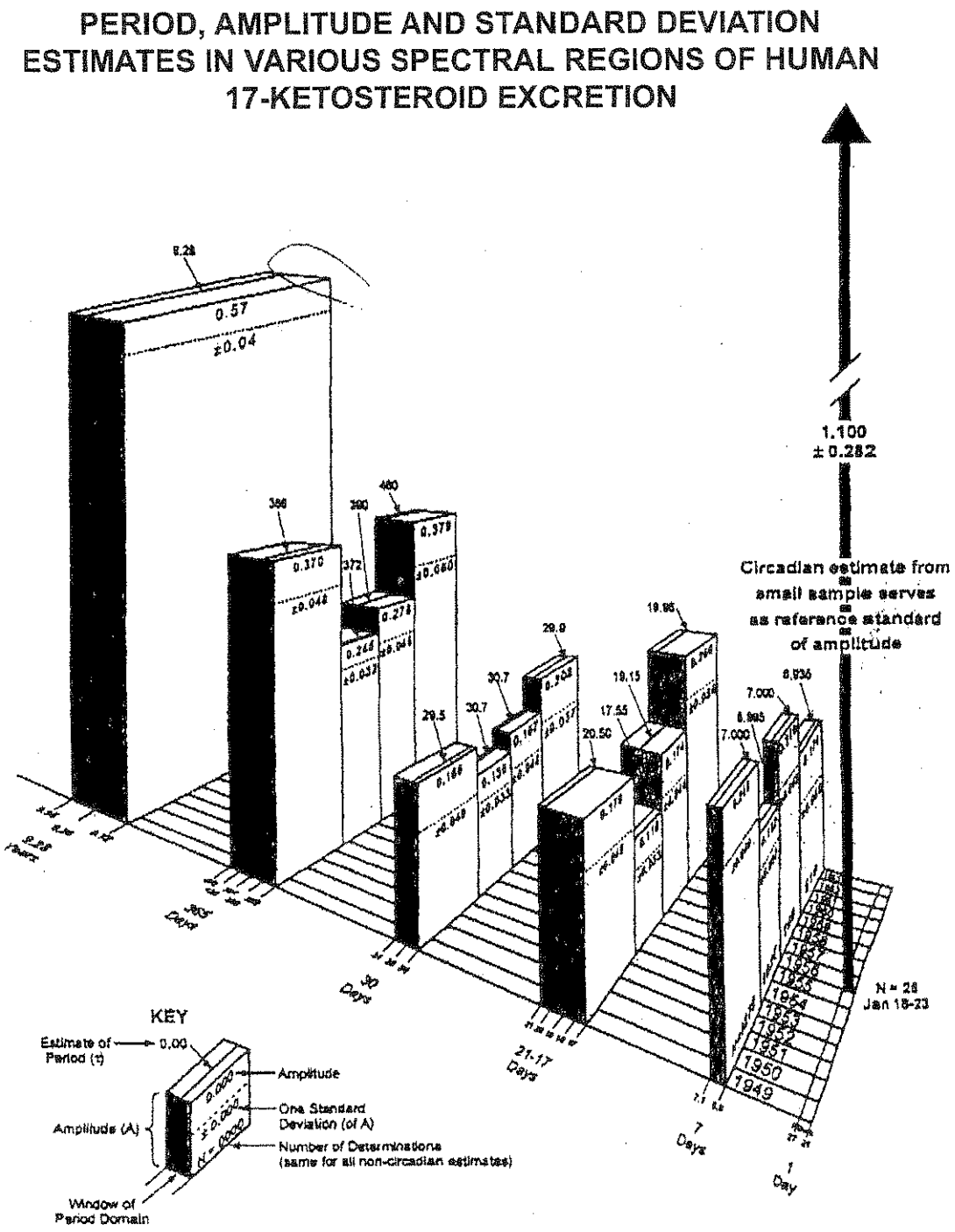


Figure 15a. Marked difference in circadian amplitude of certain (c-Fos) immunoreactive cells in two different parts of the suprachiasmatic nucleus (SCN) on two lighting regimens.

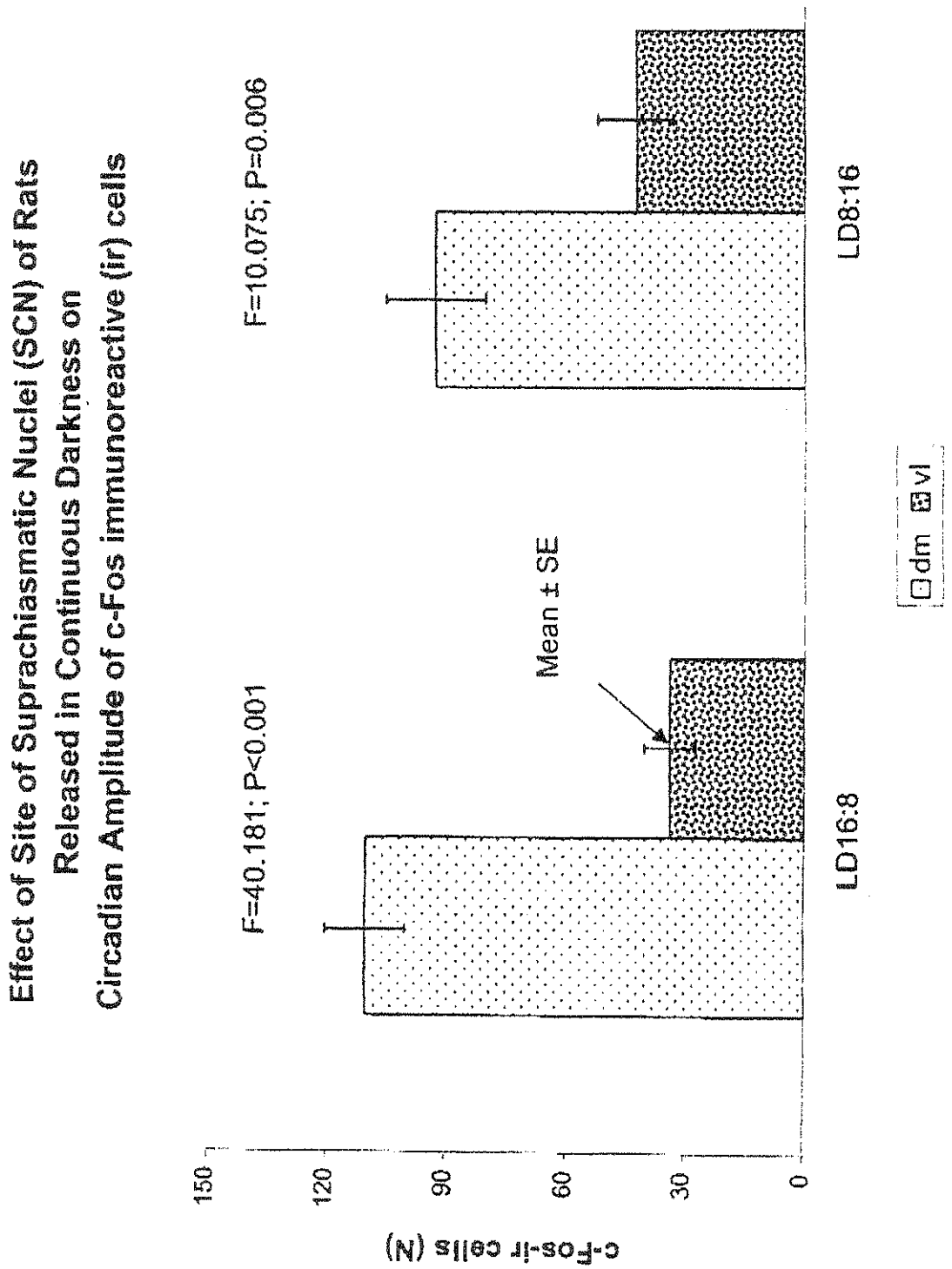


Figure 15b. When values are expressed as a percentage of the mean (since the means were different, not shown), the difference in the circadian amplitude of c-Fos cells as a function of site is apparent in the LD16:8 regimen only.

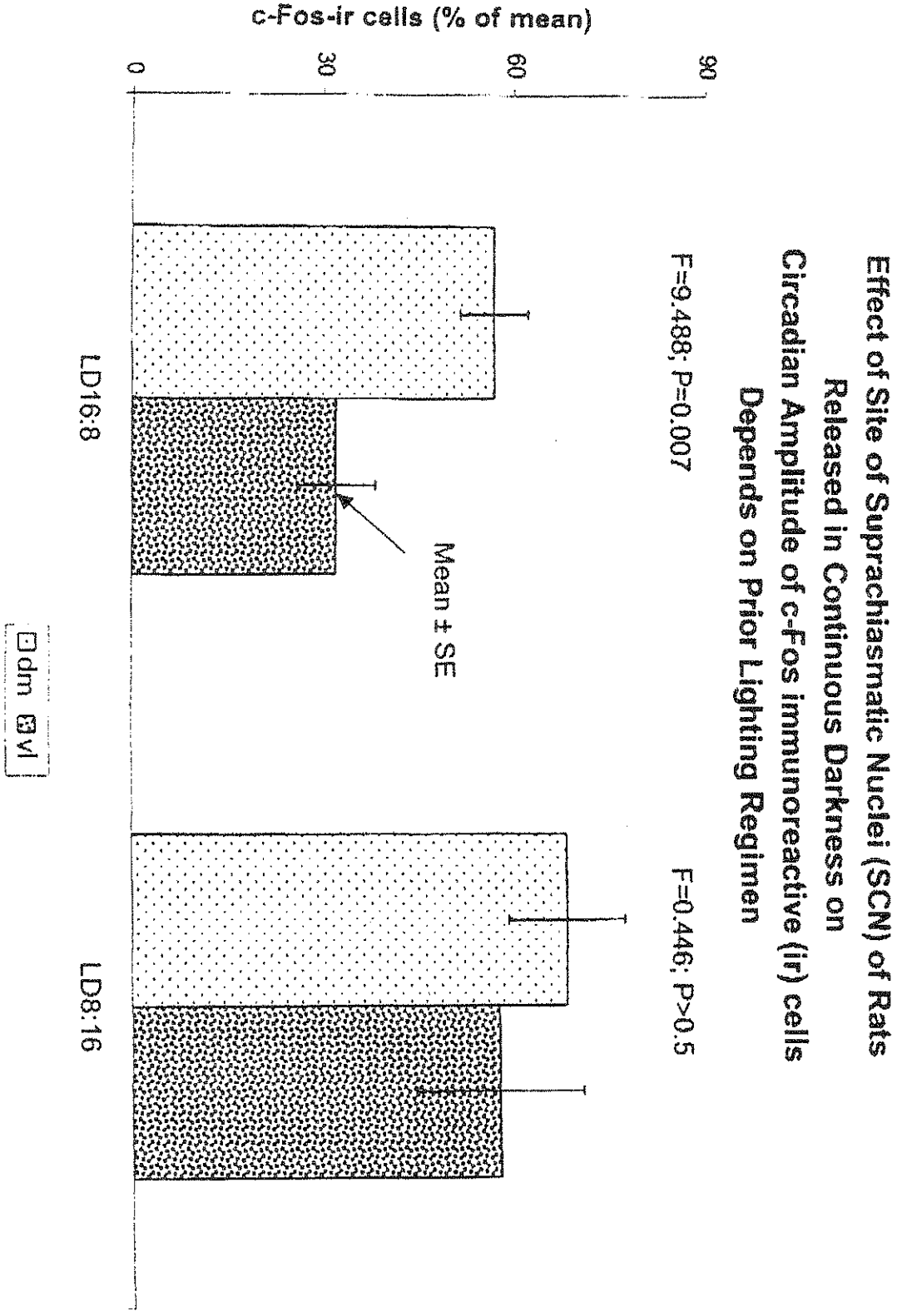


Figure 15c. The larger amplitude in the dorsomedial as compared to the ventrolateral part of the SCN, seen on LD16:8 (top) but not on LD8:16 (bottom), when expressed in relative terms summarized in Figure 15b, is here shown as a larger swing in the time domain, supporting the possibility of an amplification by added light in the dorsomedial SCN.

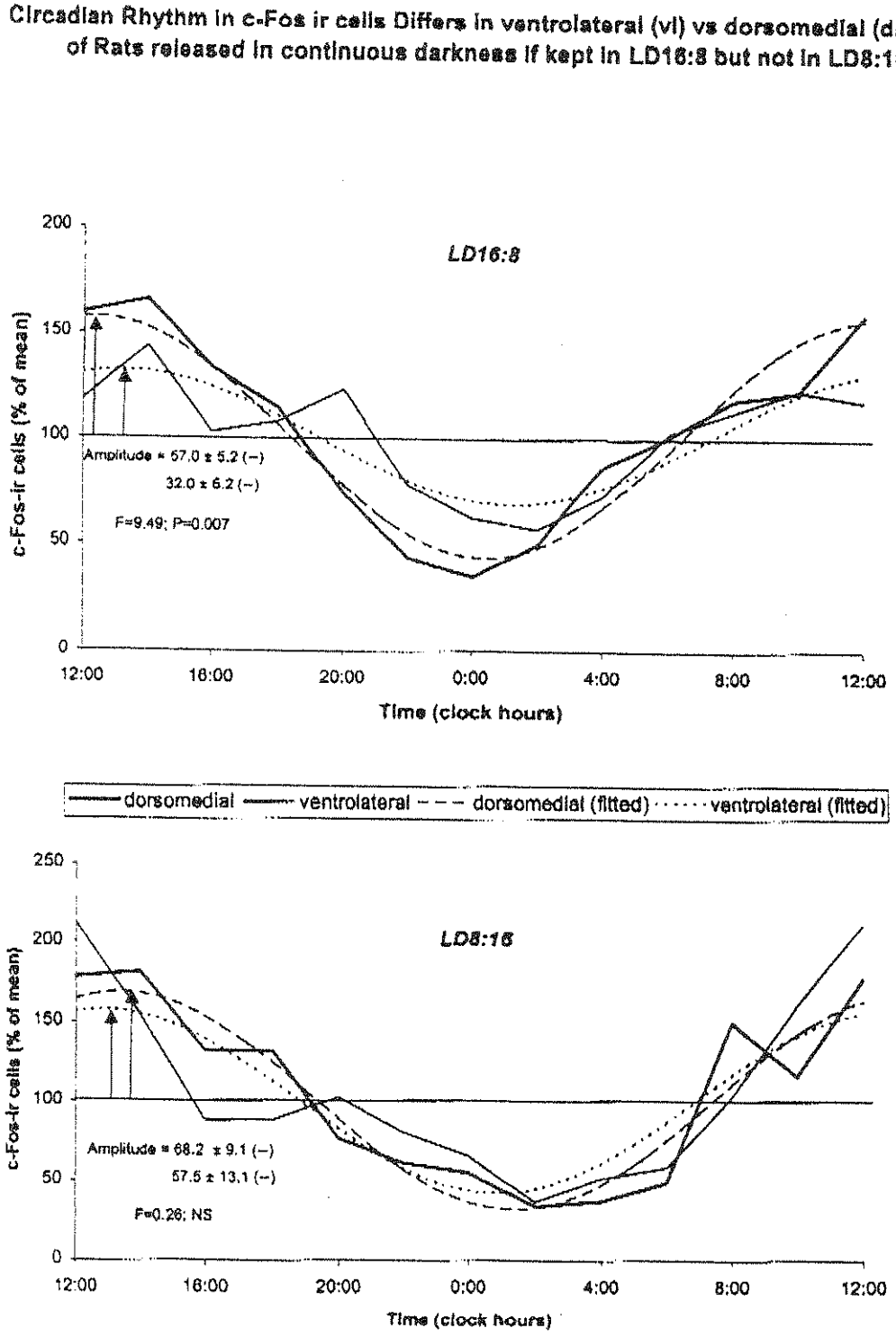


Figure 16a. During a minimum of the ~10.5-year Schwabe cycle of solar activity, a circadian rhythm is found in a group of newborns with a positive (top) but not in one with a negative family history (bottom) of high blood pressure and/or other vascular disease.

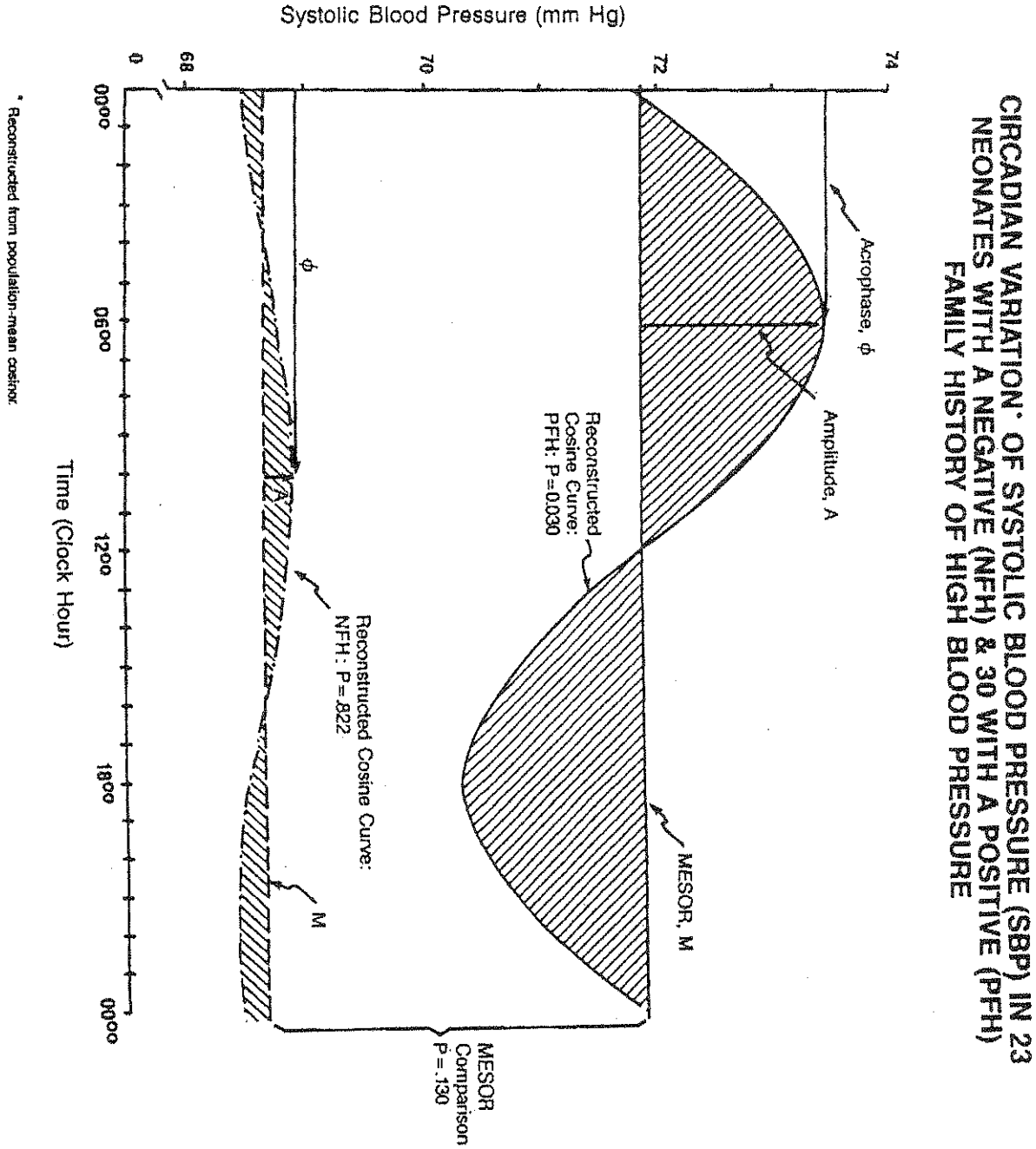
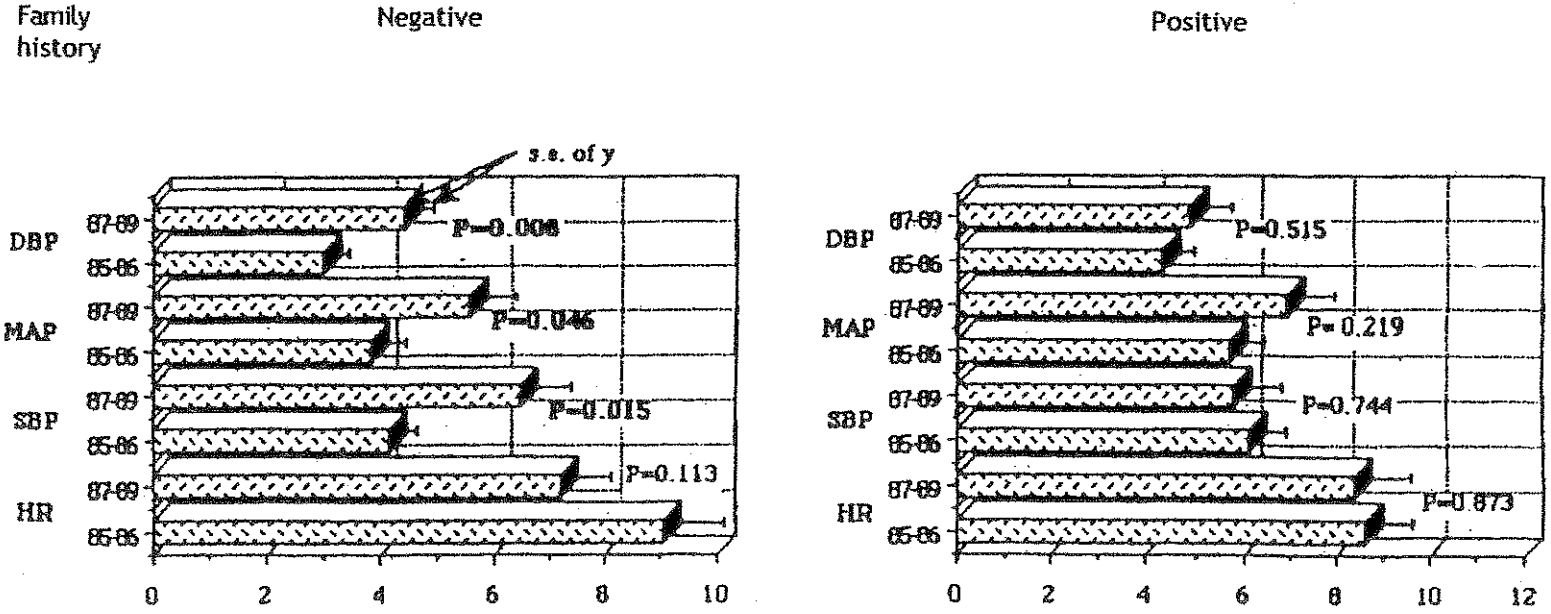


Figure 16b. Changes in circadian amplitude of blood pressure with solar cycle stage, in the absence of concomitant changes in the chronome-adjusted mean or MESOR, the latter not shown.

INCREASE IN CIRCADIAN DOUBLE AMPLITUDE (A) OF BLOOD PRESSURE (BP) BUT NOT HEART RATE (HR) OF NEWBORNS WITH A NEGATIVE (left) BUT NOT WITH A POSITIVE (right) FAMILY HISTORY OF HIGH BLOOD PRESSURE* WITH ASCENDING SOLAR CYCLE STAGE



Units: BP: mm Hg; HR: beats/min

*And/or other vascular diseases.
S: systolic; MA: mean arterial; D: diastolic; HR: heart rate. *Assessed by comparison between results obtained in solar minimum (1985-1986) vs. ascending stage of solar activity (1987-1989) by means of Student's t-test.

Figure 16c. A clear circaseptan pattern with an appreciable extent of change is summarized based on 48-hour profiles at 30-minute intervals from infants studied during different 48-hour spans of the first week of their life.

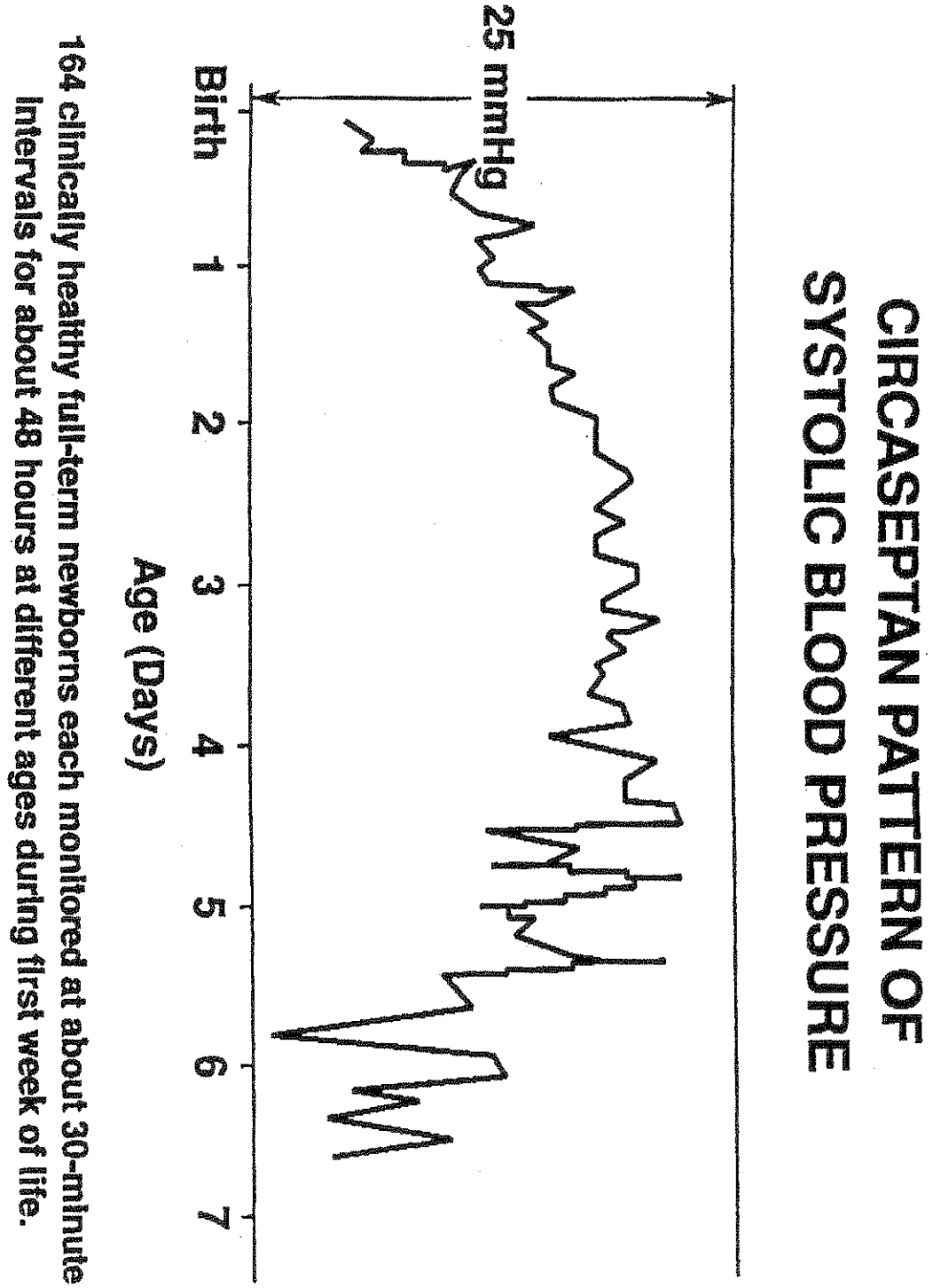


Figure 16d. Differences as a function of family history of high blood pressure and other vascular disease along the scale of the first 7 days of life are shown time-macroscopically.

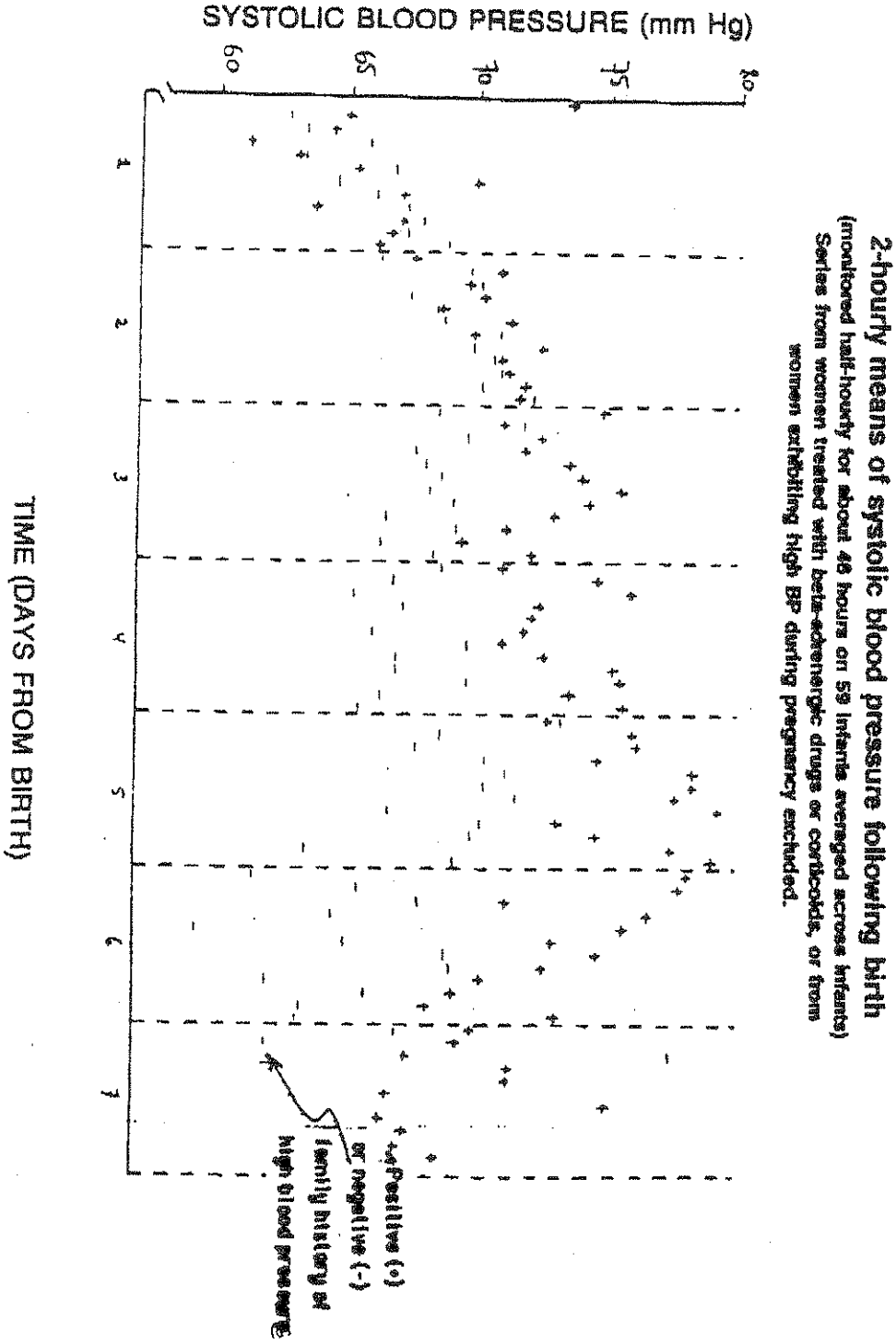
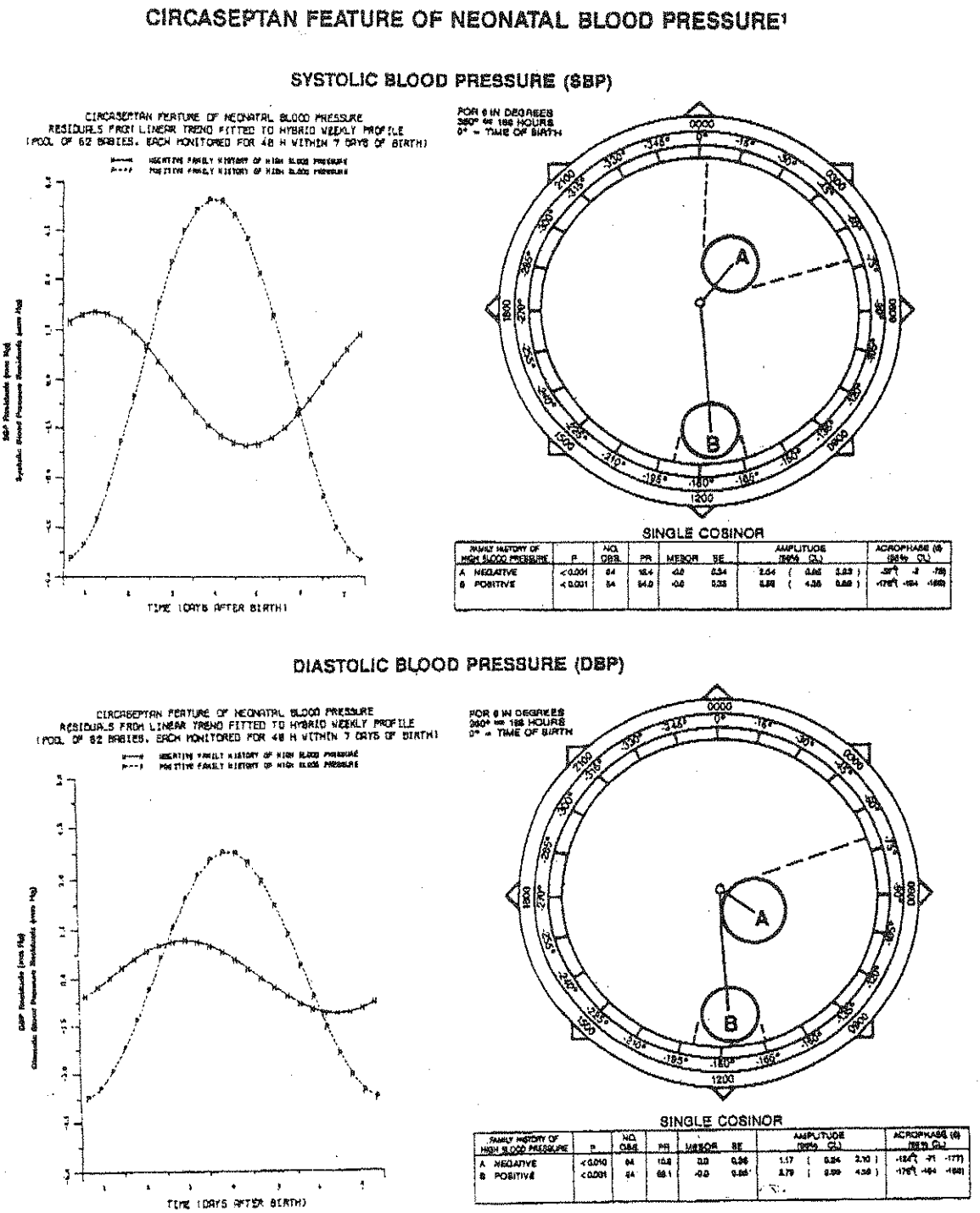


Figure 16e. Time-microscopy by cosinor confirms a statistically significantly different circaseptan rhythm as a function of the family history of high blood pressure and/or vascular disease, a problem to be checked for a dependence upon solar cycle stage when blood pressures are examined longitudinally rather than for 48 hours for earliest vascular disease risk detection, a problem still unsolved.



PHOTOPERIODIC MODULATIONOF CLOCK AND CLOCK-CONTROLLED GENES EXPRESSION

Helena Illnerová, Martin Jáč, Alena Sumová

Institute of Physiology, Academy of Sciences of the Czech Republic,
142 20 Prague 4, Czech Republic

Address correspondence to:

Doc. RNDr. Helena Illnerová, DrSc.
president of The Academy of Sciences of the Czech Republic
Národní třída 3
117 20 Praha

Mammalian circadian rhythms are controlled by a pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Morphologically and physiologically, the SCN is composed of a ventroloteral (vl), retinorecipient part, and of a dorsomedial (dm) part. Eight clock genes, their expression and production of corresponding proteins form intervening negative and positive feed-back loops creating thus a circa 24-h core clock oscillations. Clock genes expression affects also clock-controlled genes expression. Clock and clock - controlled genes are expressed rhythmically not just in the SCN, but also in peripheral organs, such as is the heart, liver, lung, skeletal muscle, testic etc. Light and the photoperiod may entrain the SCN circadian rhythmicity via c-fos gene expression and CREB phosporylation.

The aim of the present study was to find out whether and how the photoperiod affects the entraining pathway in the rat vl-SCN, namely the rhythm in c-fos gene photoinduction, further the rhythm of the clock-controlled gene expression, namely of arginine vassopressin gene expression, in the dm-SCN, and finally the rhythm of clock gene, namely of Per1, expression in the whole SCN. In rats maintained in long summer days, the interval enabling c-fos gene photoinduction in the vl-SCN was shorter than that in rats maintained in short winter days. The interval of elevated AVP gene expression in the dm-SCN as well as the interval of elevated PER1 protein production in the whole SCN was longer in rats maintained in long summer days than in those maintained in short winter days. Importantly, PER1 production occurred mostly in the dm-SCN of the rat. Altogether, the data show that the rhythmic photosensitivity of the vl-SCN as well as the rhythmic clock and clock-controlled genes expression in the dm-SCN are affected by the previous photoperiod. As the photoperiod modulates rhythmicity of the vl-SCN as well as of the dm-SCN, it modulates the whole SCN rhythmicity and presumably the rhythmicity of the whole ogranism.

BLOOD PRESSURE AND HEART RATE DYNAMICS DURING PREGNANCY AND EARLY EXTRA-UTERINE LIFE: METHODOLOGY FOR A CHRONONEONATOLOGY

Germaine Cornelissen, Jarmila Siegelova*, and Franz Halberg

University of Minnesota, Minneapolis, Minnesota USA
*Masaryk University, Brno, Czech Republic

Address correspondence to:

Germaine Cornellissen,Ph.D.,
Co-Director, Director of Chronobiometry,
Halberg Chronobiology Center,
University of Minnesota
715 Mayo Building
Mayo Mail Code 8609
420 Delaware St. S.E.
Minneapolis, MN 55455, USA

*Invited lecture on chronomics at Masaryk University, Brno, Czech Republic

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ABSTRACT

The merits of longitudinal monitoring and of normalization procedures are illustrated for the case of blood pressure and heart rate data collected during pregnancy and early extra-uterine life. By reducing the inter-individual variability, we map with point and interval estimates the known course of a healthy, uncomplicated pregnancy, with blood pressure reaching a minimum during the second trimester and heart rate increasing throughout pregnancy. Well within an otherwise neglected physiological range of acceptable values, however, we show a difference of about 8 mmHg separating healthy pregnancies from pregnancies complicated by gestational hypertension and/or pre-eclampsia. An excessive circadian amplitude of blood pressure, a risk factor for cerebral ischemic events and nephropathy in the general population (irrespective of an elevated blood pressure and the presence of other risk factors), predicted severe pre-eclampsia with a lead time of 8 weeks. In neonates, a circadian rhythm is detectable shortly after birth, when it peaks during the night, with a timing opposite to that found for the mother. During the first 2 to 3 weeks of life, the circadian rhythm and its prominence within the broader chronome is changing. Its amplitude is getting progressively larger, and its acrophase is adjusting to mid-afternoon hours. This adjustment can differ among babies, being accomplished by phase shifts and/or drifts. Ultradians, with a period of 3 to 4 hours synchronized by the feeding schedule are also detected in early extra-uterine life. Immediately after birth, the circaseptan component, with a period of about one week, is usually more prominent than the circadian rhythm and any ultradian component. Circaseptan timing is determined primarily by the time of birth. The circaseptan period of diastolic blood pressure and heart rate appears to be inherited, as shown by intra-class correlation analysis on twins. During the first week(s) of life, the heart rate increases, and girls tend to have a higher blood pressure than boys, trends that reverse later on. Already at birth, the circadian and circaseptan amplitudes can separate groups of babies with a positive or negative family history of high blood pressure and/or related diseases. Whereas this difference can not always be validated with statistical significance, it is in keeping with results obtained in groups of older children, and in adulthood. Babies who have been exposed to betamimetic drugs in utero tend to have a larger circadian amplitude of blood pressure, a finding persisting into adolescence. These results, observed within the usually neglected physiological range, illustrate the merits of chronomics, the mapping of time structures (chronomes) in health, so that early alterations can identify an elevated disease risk and prompt prophylactic intervention to be instituted in a timely fashion.

INTRODUCTION

Pre-eclampsia is a common disorder of pregnancy, characterized by high blood pressure and proteinuria, and posing a serious health risk to both mother and fetus (Berman et al., 2001). Oxidative stress is a prominent feature of the placenta in many complications of pregnancy, such as pre-eclampsia. The cause is primarily unknown, although ischemia-reperfusion injury has been considered to be one possible mechanism (Hung et al., 2001). A disturbed growth of throphoblast cells, probably induced by an altered maternal immunotolerance has also been proposed

(Wolf et al., 2001). Several risk factors and/or biomarkers have been proposed, such as an excessive placental secretion of neurokinin B during the third trimester of pregnancy (Page et al., 2000), alterations of plasma calcium and intracellular and membrane calcium in erythrocytes (Kosch et al., 2000), hyperhomocysteinemia (Herrmann, 2001), reduced concentrations of angiopoietin-2 mRNA (Zhang et al., 2001), increased AT(1) receptor heterodimers, mediating an enhanced angiotensin II responsiveness (AbdAlla et al., 2001), vascular endothelial growth factor (El-Salahy et al., 2001), endothelin-1 (Singh et al., 2001), and selectins (Acar et al., 2001).

Thought to be a serious multi-system disorder with general endothelial disease, the pathogenesis of pre-eclampsia remains, however, not fully understood (Dekker and Sibai, 1998), and no specific diagnostic tests are available for early and reliable diagnosis, or for monitoring of the disease process (Berg et al., 2001). Diagnosis of pre-eclampsia and of other complications of pregnancy requires an understanding of the physiological adaptations during healthy uncomplicated pregnancy. This is best achieved by monitoring vascular variables and by analyzing their time structure (chronome). Mild pre-eclampsia has been reported to be associated not only with elevated plasma norepinephrine concentrations, but also with lower systemic daytime production of prostacyclin, and with blunting of the usual circadian variation in a number of variables, such as plasma concentrations of BNP, ANP, noreprinephrine, and aldosterone, as well as urinary prostacyclin metabolites (Kaaja et al., 1999; see also Miyamoto et al., 1988, and Beilin et al., 1982).

Herein, focus is placed on changes in blood pressure and heart rate, resolved inside the physiological range to provide refined reference values, in the light of which early alterations may become indicative of an increased vascular disease risk and prompt the timely institution of prophylactic intervention. From the mapping of the circadian variation in blood pressure and heart rate as it changes during the course of pregnancy, differences well within the usual value range are illustrated between healthy and complicated pregnancies. After delivery, we show that a circadian rhythm is also detectable in neonates, that is in anti-phase with the maternal pattern, and that both ultradian and infradian components (with frequencies higher and lower than one cycle per day, respectively) are also present. In particular, the circaseptan component (with a period of about a week) is prominent immediately after birth, and its period seems to be inherited, as suggested by intra-class correlation analysis on premature twins.

STUDIES IN PREGNANCY

Circadian Reference Standards

Between July 1988 and July 1990, 223 pregnant women in Minnesota, USA, 20 to 40 years of age, provided 362 profiles of blood pressure and heart rate measurements at 1-hour intervals over 48-hour spans, obtained with the ABPM-630 ambulatory monitor from Colin Medical Instruments (Komaki, Japan). At the outset, most women were clinically healthy with no known elevation in blood pressure. Measurements from pregnant women with a clinical diagnosis of an elevated blood pressure made later in pregnancy were excluded for the

computation of time-specified reference standards (chronodesms). The latter were derived by calculating time-specified 90% prediction limits on the pool of all profiles collected during each trimester of pregnancy, according to the technique described by Nelson et al. (1983). These limits account for both inter- and intra-individual variability. They were obtained by considering an interval of 1 hour, progressively displaced along the 24-hour scale. In any one trimester of pregnancy, the changes accounted for by the circadian variation far exceed the changes observed during the course of gestation (Cornelissen et al., 1991; Cornelissen and Halberg, 1994).

Changes during Gestation

A plot of the circadian rhythm-adjusted mean value (MESOR) of all profiles as a function of gestational age reveals a large scatter with no apparent structure, as illustrated in Figures 1-3 for the case of systolic and diastolic blood pressure and heart rate, respectively. Accounting for inter-individual variation by a simple normalization procedure (Cornelissen et al., 1989) uncovers changes occurring during pregnancy that are best followed-up longitudinally (Ayala et al., 1994). Specifically, profiles contributed by the same pregnant women at different gestational ages were paired to reduce the inter-individual variation. Not all women monitored themselves at the same gestational ages. Although changes between consecutive profiles may not have been linear, such linearity was assumed as a first approximation to estimate the rate of change per week in blood pressure and heart rate for each woman. These estimated rates of change per week were assigned to each gestational week covered by the span elapsed between the two monitoring profiles. After repeating the same procedure for all paired profiles of all women, the rates of change per week were averaged for each gestational week across all women, and integrated over the course of gestation. Results are illustrated in Figure 4. A gradual increase in heart rate is readily seen, whereas blood pressure decreases between the 12th and 15th weeks, reaching a minimum in the second trimester of pregnancy, around the 20th gestational week, followed by an increase between the 30th and 32nd weeks.

These results are consistent with reports by others. The steady increase in heart rate throughout gestation is noted, for instance, by Edouard et al. (1998) and by Hohmann and Kunzel (1999). Schulz et al. (1994) note the lowered blood pressure during pregnancy and reported a minimum reached in the second trimester. Palmer et al. (1999) also report a decrease in blood pressure until week 20 in healthy pregnancy, but noted an effect of altitude, observing this pattern at 1260 but not at 3100m. Hohmann and Kunzel (1999) find mean arterial pressure to decrease during the first two trimesters and to increase to pre-conceptual values during the last weeks of gestation. A steady decrease in blood pressure up to 20 weeks, followed by an average 8% increase between the middle of gestation and delivery is also reported by others (Hermida et al., 2001). Strevens et al. (2001) report similar results for a Swedish population studied on the basis of mercury sphygmomanometer measurements at each antenatal visit. These authors further note an effect of parity, the increase in blood pressure in the second half of gestation being greater for nulliparae than for multiparae.

Deviations from such courses have been observed in the presence of complications of pregnancy (Hermida et al., 2001). Whereas Eskes (2000) notes that nearly 40% of hypertensive patients showed drops in blood pressure in mid-pregnancy, a lack of blood pressure decrease during the first half of pregnancy is noted in women with pre-eclampsia (Palmer et al., 1999). This difference in blood pressure trend during gestation provides a basis for the MAP-2 test that Öney and Kaulhausen (1983) had proposed as a predictor of pregnancy-induced hypertension and pre-eclampsia. In relation to heart rate, Clapp (1985), who studied women longitudinally starting before conception, reported a rise of 8 beats/min by the 8th week of gestation, as compared to an overall pregnancy-associated increase of 16 beats/min, together with other changes in body composition, cardiopulmonary and metabolic function, that he found to be absent in women who later experienced spontaneous abortion (Clapp et al., 1988).

Early detection of abnormality

When the blood pressure and heart rate profiles of all pregnant women were classified as a function of pregnancy outcome (uncomplicated versus complicated by gestational hypertension or pre-eclampsia), differences came to the fore on a group basis, already during the first trimester of pregnancy. As shown in Figure 5, differences in mean arterial pressure of the order of 8 mmHg are found that are well within the range of currently acceptable values between women who are clinically healthy at the outset but will develop gestational hypertension or pre-eclampsia, and those whose pregnancy will remain uncomplicated. Similar results are found for systolic and diastolic blood pressure, with mean values below 125/75 mmHg. Such elevations in blood pressure, occurring well within the physiological range, are observed on a group basis already during the first trimester of pregnancy, when they cannot be picked up by casual measurements. Differences of the order of 8 mmHg, such as those observed in this case, may be close to the limits of the technical error for single measurements. When they stem from around the clock profiles obtained automatically with an ambulatory monitor, however, such differences have both statistical and clinical significance.

Differences in the circadian amplitude of blood pressure are also documented between healthy and complicated pregnancies, Figure 6. Women who will develop gestational hypertension or pre-eclampsia later on have a circadian blood pressure amplitude larger than healthy women with an uncomplicated pregnancy. By cosinor analysis of 24-hour blood pressure profiles from 32 patients with pre-eclampsia and 21 hypertensive patients with superimposed pre-eclampsia, Schachinger et al. (1992) found no statistically significant difference in circadian blood pressure variability. In a comparison of patients with pre-eclampsia with healthy control subjects, however, Bellomo et al. (1995) report a numerically larger day-night difference for women with the disease. A statistically significantly larger circadian blood pressure amplitude in complicated vs. healthy pregnancies in all trimesters is also reported by Hermida et al. (2000).

A case report with an unusually large circadian blood pressure amplitude in the 19th gestational week, preceding pre-eclampsia by 8 weeks, is illustrated in Figure 7. By conventional criteria, only 3 measurements were above 140/90 mmHg. Chronobiologically, the 24-hour rhythm-adjusted mean values (MESORs) of both

systolic and diastolic blood pressure were also well within acceptable limits (115/64 mmHg), but the double circadian amplitude of systolic blood pressure exceeded the upper limit of acceptability by almost 8 mmHg. Reference limits for clinically healthy non-pregnant women were used originally, because, at the time, no reference standards during pregnancy, developed in the interim (Carandente et al., 1990; Cornelissen et al., 1991; Benedetto et al., 1996) were available as yet. Reference limits later derived from 117 clinically healthy Minnesotan women who had provided 48-hour profiles in their second trimester of pregnancy eventually confirmed the diagnosis of systolic CHAT (Circadian Hyper-Amplitude-Tension). In non-pregnant women and men, CHAT has been shown to be an independent risk factor, associated with a large increase in the risk of adverse vascular events, stroke and nephropathy in particular (Otsuka et al., 1997; Halberg et al., 1998; Cornelissen et al., 1999). An elevated circadian amplitude of blood pressure preceding pre-eclampsia may perhaps account for the reported increased risk of cerebral infarction during the puerperium seen primarily after eclampsia (Mas and Lamy, 1998).

A circadian amplitude of blood pressure larger in patients with pre-eclampsia than in healthy uncomplicated pregnancies is at variance, however, with several other studies. Beilin et al. (1982) reported nocturnal hypertension in four pre-eclamptic women, who also showed a reversed circadian rhythm for noradrenaline sulphate. Miyamoto et al. (1988) found an acrophase near midnight for both blood pressure and atriopeptin in pre-eclamptic women. Öney and Meyer-Sabellek (1990) report a blunted circadian variation of blood pressure in patients with pre-eclampsia, whereas the women with severe pre-eclampsia these authors studied showed a reversed circadian rhythm. Further work is needed to determine the reasons underlying such discrepancies. One possible explanation relates to whether blood pressure was monitored before or after the diagnosis of pre-eclampsia. Conceivably, an elevated circadian blood pressure amplitude may be a precursor, which may be followed, as the condition progresses, by further alterations of the circadian pattern of blood pressure and other related variables. Another line of investigation may consider the fact that pre-eclampsia may lead to a multi-organ failure, thus involving different mechanisms of action. Of interest is a study by Kilpatrick (1996), who reports that an HLA-dependent TNF secretory response may provide an immunogenetic link between pre-eclampsia and insulin-dependent diabetes mellitus. Some patients with diabetes, indeed, have been found to show a reversed blood pressure circadian rhythm (Herpin et al., 1991).

STUDIES IN NEONATES

Expression of Circadian Rhythm at Birth

Kellerova (1981) first documented a circadian rhythm in the blood pressure of neonates. A relatively fast development of the circadian blood pressure rhythm is observed in newborns with a genetic background of high blood pressure: the slope of a linear trend and the circadian amplitude, but not the mean value of blood pressure, may separate newborns with a negative from those with a positive family history of high blood pressure during the first few days of life (Halberg et al., 1986). The statistical significance of the difference in circadian blood pressure

amplitude seems to be subject to other influences, which await resolution, perhaps by the assessment of components with much lower frequencies, mapped on a population basis.

In cooperation with the late Brunetto Tarquini of Florence, Italy, we monitored 164 newborns at 30-min intervals for 48 hours during the first week of life. The individual data series are very noisy and a circadian pattern cannot readily be visualized by displaying the data as a function of time. With a combination of single and population-mean cosinor analyses, a circadian rhythm can be demonstrated with statistical significance for blood pressure and for heart rate on a group basis ($P<0.001$) (Halberg et al., 1990), Table 1. It can be seen that, overall, high values tend to recur in the early morning hours, shortly after midnight, in contrast with high values usually found around mid-afternoon later in life, and also in the newborns' mothers before and after delivery (Schuh et al., 1989), Figure 8.

The presence of a circadian blood pressure rhythm at birth has been questioned by some authors (e.g., Garcia et al., 1995). We show that the failure to demonstrate a circadian rhythm in early extra-uterine life may stem, at least in part, from ignoring the evolving circadian pattern during the first few weeks of life. During this span, the acrophase is changing (from being in almost antiphase to being almost in phase with the maternal rhythm). Because different babies adjust their circadian pattern in different ways, an analysis pooling data over too long an interval from several infants is likely to miss the circadian variation, because the individual rhythms do not necessarily remain synchronized among the different babies. This observation is in keeping with the report by Dimitriou et al. (1999) of a circadian rhythm present on day 2 but not on day 7 after birth.

In Table 2, we show that by limiting the data to the first two days of life, a circadian component can be demonstrated with statistical significance for a group of 49 babies monitored at 20-min intervals for up to 3 weeks, starting shortly after birth. As anticipated, the circadian acrophase is found to occur in the early morning, as in previous studies. When similar analyses are carried out for subsequent 2-day spans, a circadian variation can no longer be validated on a group basis. The individual longitudinal record in Figure 9 illustrates that the changing circadian pattern may underlie the failure to detect a circadian rhythm on a group basis after the first few days of life. A summary of data collected during days 1-4, stacked over an idealized 24-hour scale, shows a statistically significant circadian variation with high values between 02:00 and 08:00 and low values between 14:00 and 20:00. Another summary of data collected during days 6-9 also detects a circadian rhythm, but with very different characteristics, high values now occurring between 12:00 and 18:00 and low values between 00:00 and 08:00. By pooling all data in a single analysis, the circadian rhythm cannot be validated. The changing circadian characteristics, assessed by chronobiologic serial section, are shown in Figure 10. In addition to an increasing mean value, a relatively abrupt change in acrophase is seen to occur between the 5th and 6th days of life. Not all babies adjust their acrophase in the same way. Another record shown in Figure 11 illustrates that the acrophase can slowly drift. As a consequence, the circadian rhythm may have a period differing from 24 hours. Moreover, the circadian period may change, being approximately 27.3 hours during days 1-5 and slowing down to about 25.6 hours during days 14-18. ***Circadian Blood Pressure Pattern and***

Vascular Disease Risk

As shown in Table 1, even though the amplitude of the circadian blood pressure rhythm at birth is very small, it can differ on a group basis between neonates with a positive or negative family history of high blood pressure and/or related vascular disease. This difference was most prominently seen at the beginning of the study conducted in 1985-86 and could not be statistically validated in data collected in 1987-88 considered separately. Such a difference in the pattern of the circadian rhythm in blood pressure is in keeping with results observed in older children (for review, see Cornelissen et al., 1993; Halberg et al., 1993). In adulthood, an above-threshold circadian blood pressure amplitude has been shown to be an independent risk factor associated with a large increase in vascular disease risk (Otsuka et al., 1997; Halberg et al., 1998; Cornelissen et al., 1999, 2001). Of further interest is the higher degree of classification obtained by combining indices of variation in blood pressure and heart rate: for the first set of 53 neonates studied, a 72.3% equivalent classification was reached based on the circadian amplitude of diastolic blood pressure, the 50% range of systolic blood pressure, and the standard deviation of heart rate (Halberg et al., 1987). In adulthood as well, we showed that a below-threshold heart rate variability, gauged by the standard deviation, was a vascular disease risk factor independent of the risk associated with an above-threshold blood pressure variability, gauged by the circadian amplitude (Cornelissen et al., 2000).

The range of the predictable within-day change in blood pressure, assessed as the circadian blood pressure amplitude, is also greater in the 39 newborns who had been exposed *in utero* to betamimetics than in the 126 babies not so exposed, Figure 12 (Halberg et al., 1990). This altered pattern of the circadian blood pressure rhythm was interpreted as a potential increase in vascular disease risk associated with betamimetic exposure in the womb. A study in older children by Syutkina et al. (1995) concluded that the effects of exposure to betamimetics *in utero* lasted into adolescence, and involved with the circadian blood pressure amplitude also the left ventricular mass index. Adrenergic mechanisms have long been claimed to be responsible for cardiac damage, as reviewed by Raab (1969). Evidence of the harmful effect of betamimetic drugs on the fetal heart had been presented by Karlsson et al. (1980), whereas Crawford et al. (1982) reported a thickening of the interventricular septum in infants exposed *in utero* to ritodrine, and Nuchpuckdee et al. (1986) described the occurrence of cardiac septal hypertrophy after *in utero* ritodrine exposure. More recently, Gokay et al. (2001) reported that ritodrine treatment may alter placental and cerebral blood flow and may have a selective effect on the left side of the heart. Potential neonatal myocardial injury from long-term tocolysis was also reported by Friedman et al. (1994), Wieczorek (1996) and Adamcova et al. (2000).

Expression of Circaseptan Rhythm at Birth

Between August 1985 and June 1986, when the blood pressure and heart rate of 62 clinically healthy full-term newborns of both genders had been measured automatically around the clock at about 30-minute intervals for 48 hours during the first week of life (Cornelissen et al., 1987), a first glimpse of the circaseptan variation came to the fore. This was achieved by pooling data across neonates and

by using a normalization procedure, since each series covered only two days. The data were referred to the time of birth. They were averaged in consecutive 2-hour bins, separately for each infant. By expressing the data as a function of age rather than as a function of the day of the week, any environmental and/or lingering maternal synchronization and/or any possible contribution of behavioral differences among babies born at different circadian stages were thus (by necessity) ignored. The normalization procedure consisted of considering the differences between consecutive 2-hour averages for each individual data series, which was assigned to the mid-point of the corresponding 4-hour interval in relation to the baby's time of birth. It was thus possible to minimize the effect of inter-individual differences in MESOR among newborns and the contribution of relatively large ultradian variability. Since different babies started monitoring at different times during the first week of life, the relative changes thus computed spanned roughly the first week post-partum once all the data are pooled. The global changes taking place during the first week of life were reconstituted by averaging across babies providing data at a given time-point the 2-hourly differences pertaining to a given post-birth interval, by using as starting value the mean of all original data collected within the first 6 hours of life, and by integrating consecutive averaged differences over time. The resulting data series can be modeled by a 7-day cosine curve superimposed upon a linear trend ($P<0.001$). After increasing during the first 3 days of life, blood pressure decreases for the next few days. When the data contributed by neonates with a positive or negative family history of high blood pressure are analyzed separately, a statistically significantly larger circaseptan amplitude is found to characterize newborns with a positive family history (2A = 11.4/7.9 vs. 3.2/3.0 mmHg SBP/DBP; $P<0.01$) (Cornelissen et al., 1987).

The first longitudinal validation of a circaseptan component of blood pressure and heart rate came from a very premature Caucasian boy born prematurely at 27 weeks, hospitalized for most of the first 26 months of life. The data were available at intervals of 1 to 4 hours for the fisrt few weeks after birth. As shown in Figure 13, the circaseptan component of systolic blood pressure has an amplitude almost twice as large as the circadian rhythm. Similar results apply to diastolic blood pressure and heart rate (Halberg et al., 1994). Further evidence was obtained from a study of 20 very premature babies born in Minnesota before 33 weeks, 7 of whom succumbed to SIDS (Halberg et al., 1991). Data were retrieved from hospital records during the first week of life. The circaseptan to circadian amplitude ratio for systolic and diastolic blood pressure and heart rate were 2.5, 2.6, and 7.3, respectively, all statistically significantly larger than unity ($P=0.024$, 0.020, and <0.001, respectively), corroborating the large prominence of the biologic week at birth. A circaseptan to circadian amplitude ratio larger than unity was also found in another study of 14 premature babies hospitalized in the neonatal intensive care unit of the University of Minnesota Hospital and Clinic, monitored every 1 to 3 hours for 2 to 4 weeks (Wrbsky et al., 1993), as well as in other studies in the Czech Republic (Siegelova et al., 1996) and in Russia (Turti et al., 1996). Figure 14 visualizes the circaseptan variation in blood pressure from a high-risk premature girl. The predominance of the weekly over the daily variation in systolic blood pressure is illustrated for the case of another premature high-risk infant girl in Figure 15. About-weekly changes in heart rate are shown in Figure 16 for a baby

with intraventricular hemorrhage. The results summarized in Figure 17 suggest that the circaseptan component may be particularly prominent in babies with a low birth weight. Circaseptans are also detected, however, in full-term, clinically healthy babies, as shown in Figure 18 for diastolic blood pressure recorded during the first 16 days of life. Results from another longitudinal record during the first 6 months of life fully corroborate the prominence of the circaseptan component in early extra-uterine life (Watanabe et al., 2001).

Inheritance of Circaseptan Characteristics

In the Czech Republic, blood pressure and heart rate were measured automatically at 30-minute intervals for about a week or longer from 56 premature babies in the neonatal intensive care unit (Siegelova et al., 1996). In order to visualize the circaseptan component for the group as a whole, each data series was first transformed by expressing the data as a percentage of the series mean value, which was equated to 100%. Pooled relative data from all newborns were then stacked over an idealized 7-day week. Using 7 one-day classes, one-way analyses of variance (ANOVA) tested the equality of the 7 daily means. This procedure was carried out in two ways, using two different time scales, or rather two different reference times. In the first approach, the data were assessed as a function of the day of the week (Sunday, Monday, ...). In the second approach, the time of birth was used as reference, thereby assessing changes as a function of post-natal age. Results obtained for diastolic blood pressure are illustrated in Figure 19. Whereas the biological week (data referred to the time of birth) is statistically significant (P=0.011), the corresponding social component cannot be statistically validated (P>0.20) by ANOVA. Similar results are obtained by population-mean cosinor, not only for diastolic blood pressure (Figure 19), but also for systolic blood pressure and for heart rate (not shown). A weak day of the week effect is suggested, however, by single cosinor analysis of the 7 daily mean values. The demonstration of a biological but not of a social week at birth strongly points to the genetic basis of the circaseptan component in blood pressure and heart rate.

To test for the inheritability of the circaseptan component, data from 11 pairs of Minnesotan twins recorded in the neonatal intensive care unit were obtained (Cornelissen et al., 2001). Each longitudinal data series was analyzed by nonlinear least squares. Using a trial period of 7 days, an estimate of the circaseptan period was obtained. In most cases, this component could be validated statistically by the non-overlap of zero by the 95% confidence interval for the circaseptan amplitude. Heritability was assessed by computing the intra-class correlation coefficient for the nonlinearly assessed period of each variable. When considering the 5 twin pairs that were not dizygotic, the extremely small number of twin pairs notwithstanding, the intra-class correlation coefficient differed from zero with statistical significance for diastolic blood pressure (rI = 0.957; P<0.001) and for heart rate (rI = 0.825; P=0.012), Figure 20 (Cornelissen et al., 2001).

Other Chronome Components Mapped at Birth

Ultradian components are also present in the blood pressure and heart rate chronomes of neonates. As can be seen in Figure 21 summarizing group results, the 12-hour component of systolic blood pressure is almost as prominent as the

circadian rhythm during the first week post-partum. It likely contributes to the description of a non-sinusoidal waveform of the circadian variation. In addition, a 3-hour component is statistically significant. It is particularly prominent for the case of heart rate, Figure 22. This component is likely related to the feeding schedule. Indeed, in this study, all babies were fed every 3 hours, except during one timepoint at night. In another study where babies were fed every 4 hours, a statistically significant spectral peak was found at a frequency of 1 cycle per 4 hours.

As illustrated in Figure 16, the heart rate, much higher in newborns than in older children and in adults, does not decrease immediately after birth. Heart rate continues to increase during the first few weeks of life before it reverses and starts decreasing toward adult values. An increase in the heart rate of Minnesotan twins was validated on a group basis ($t=2.900$; $P=0.010$) (Cornelissen et al., 2001). An increase in the heart rate of 11 full-term babies between the ages of 2 days and 4 weeks was also reported by Sitka et al. (1994). Heart rate was also found to increase slightly but consistently in 10 healthy growing premature infants studied by Davidson et al. (1997).

Higher blood pressure values in girls than in boys were reported by Krauss (1964), but other authors did not find any gender differences in blood pressure at birth (see for instance Report of the Second Task Force on Blood Pressure Control in Children, 1987). According to this report, gender differences become apparent only after puberty, when boys tend to have a higher blood pressure than girls. Later in adulthood, higher blood pressures in men than in women at similar ages were also reported by Reckelhoff (2001) and by Zemva and Rogel, 2001). In our study with Brunetto Tarquini in Florence, Italy, we did find a small gender difference in the 24-hour rhythm-adjusted mean value (MESOR) of blood pressure, girls having a slightly higher blood pressure than boys (Mainardi et al., 1993). In the absence of medication *in utero*, girls had a MESOR of systolic/diastolic blood pressure of 73.3/44.9 mmHg, as compared to 70.2/43.1 mmHg for boys ($P<0.05$). An inverse relationship between blood pressure and birth weight (Blake et al., 2001) is not likely to account for the gender difference found during the first week of life, since the birth weight did not differ with statistical significance between boys and girls in our study.

CONCLUDING REMARKS

Useful new information can be gained by resolving the lawful variability that takes place inside the usual value range. In the examples herein, we have illustrated a few procedures to extract information from noise, in blood pressure and heart rate data of pregnant women and newborn babies. The fact that some chronome components can be anticipated a priori, such as the circadian variation and the biological week, notably when they are synchronized with the socio-ecologic environment, contributes great statistical power to chronobiologic designs and analyses.

In view of its importance for clinical decision-making, there is an urgent need for appropriate reference ranges for chronome endpoints at all ages, the pregnant woman and her newborn baby in particular. Elevations in blood pressure MESOR associated with complications of pregnancy can be detected on a group basis

already during the first trimester of pregnancy when single measurements are uninformative. Rhythm characteristics such as the circadian amplitude of blood pressure provide new diagnostic tools in their own right, deserving follow-up, notably in the absence of any changes in mean value. Such early warning signs may be easily obtained by means of non-invasive longitudinal monitoring. The early recognition of an increased blood pressure (and proteinuria) is crucial for the prevention and/or treatment of pre-eclampsia, since this condition is completely reversible with delivery. According to a recent editorial by Roberts (2001), the pathophysiological changes of pre-eclampsia leading to maternal and fetal nortality and morbidity are largely determined before clinically evident disease, and once diagnosed, reasonably low-tech management strategies are available to prevent adverse outcomes.

The resolution of small amplitude rhythms is also important to understand the relative contributions of genetics and of the environment in shaping rhythms. While the genetic basis of circadians is no longer disputed, the results on neonatal twins herein provide new evidence for the endogenicity of the biological week as well. Its prominence in early extra-uterine life may account for the contradictory results in the literature regarding the demonstration of a circadian rhythm at birth. Mirmiran et al. (1992) deserve credit for showing that circadian rhthms are already present in the fetus. According to these authors, at a certain stage of pre-natal hypothalamic development, around 30 weeks of gestation, the fetus becomes responsive to maternal circadian signals. They also point out that the fetus is able to express circadian rhythms in the absence of maternal or environmental influences, notably in the case of heart rate.

A circadian rhythm in fetal heart rate, in phase with that of the mother, was also described in data from Theo Hellbrügge, who wrote in 1960: 'Little is known about the physiological functions before birth. One of the few data available is the heart rate. We checked twelve healthy pregnant women. From the eighth to the tenth month of pregnancy the pulse frequency and the fetal heart sounds were checked every two hours during daytime and every three hours during the night.' (Hellbrügge, 1960). A cosinor analysis of the data reveals a statistically significant circadian variation for both the fetus ($P<0.001$) and the mother ($P=0.011$), peaking around 14:08 and 13:08, respectively, but with an amplitude about 5 times smaller in the fetus than in the mother (Cornelissen et al., Halberg et al., 2001). Hellbrügge (1960) was first to point out that a circadian rhythm in heart rate was demonstrable on day 2 post-partum, but not on days 4, 6, and 8, or on weeks 1, 2, and 3, to be expressed again during weeks 6-18, and thereafter. Our results summarized herein fully confirm his original mapping of the circadian rhythm of heart rate during early human development.

The results herein also broaden the scope of a budding chrononeonatology, from circadian systems to time structures. In these chronomes, initially, built-in infradians take center stage during the first weeks of life, as a seeming survival feature of human postnatal development, documented in the Czech Republic as well as in Germany, Italy, Japan, Russia, Spain, and Minnesota (USA). Applications in the clinic and in basic science of this information await the development of miniaturized unobtrusive non-invasive instrumentation for the longitudinal monitoring of vital signs and for the as-one-goes analysis of the data thus collected. This may be particularly rewarding for the prevention of pre-

eclampsia in cases such as the one illustrated in Figure 7 accompanied by alterations in blood pressure variability, unassessed in current medical practice. An even greater challenge is the institution of countermeasures as early in life as possible to prevent diseases later in life. Strategies to reach this goal are suggested by the identification of risk already at birth. Since objective vascular alterations are observed early in life, the mapping of chronomes at all ages not only serves the double purpose of risk identification and of assessing the effectiveness of prophylactic countermeasures, but it is also indicated the earlier the better. This is the challenge of the use of available methodology in the service of chrononeonatology.

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Figure 1. Transversely, across individuals, there is a large scatter in the circadian MESOR of systolic blood pressure. If ignored, this inter-individual variability obscures changes that take place during gestation.

C41

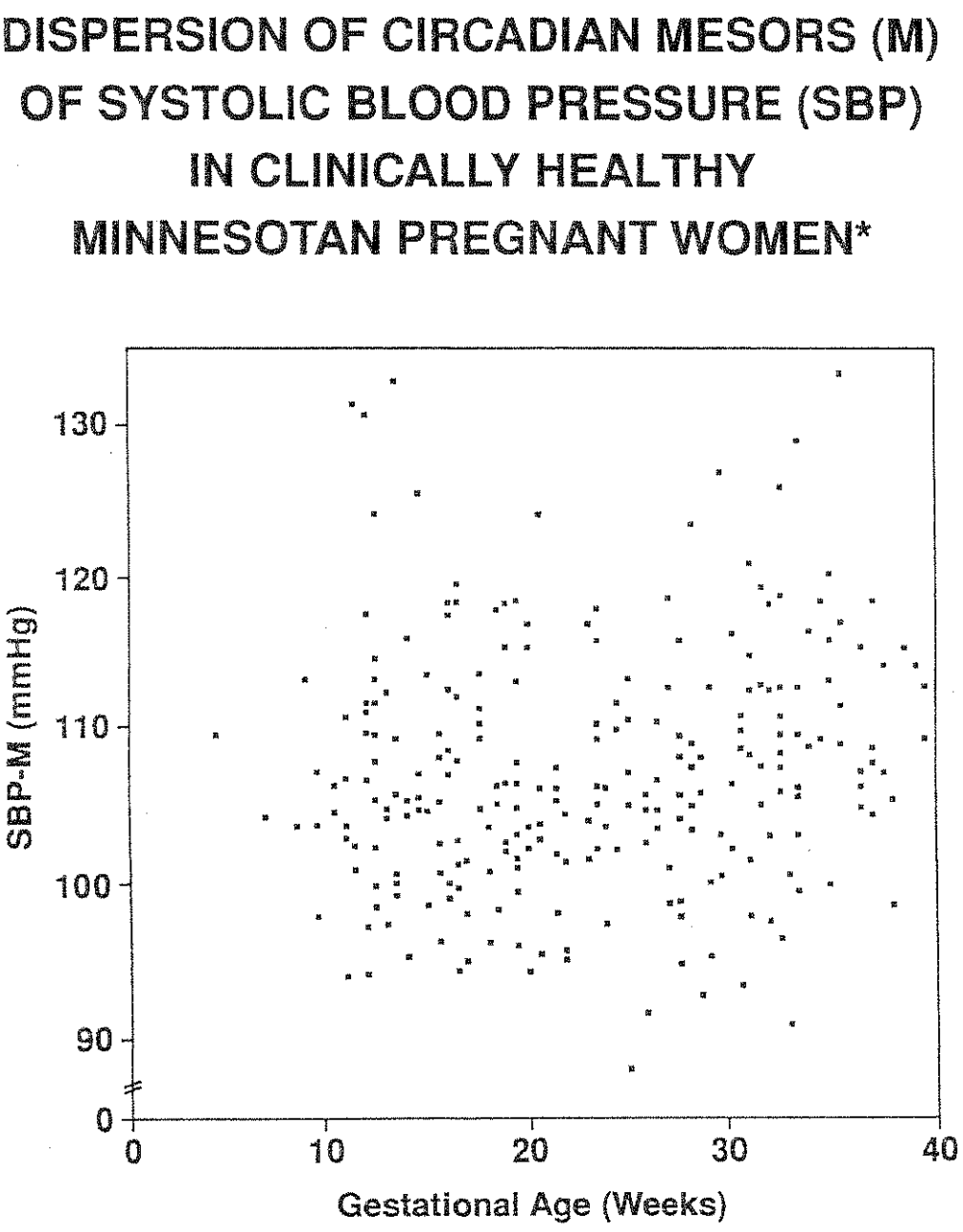


Figure 2. Transversely, across individuals, there is a large scatter in the circadian MESOR of diastolic blood pressure. If ignored, this inter-individual variability obscures changes that take place during gestation.

C43

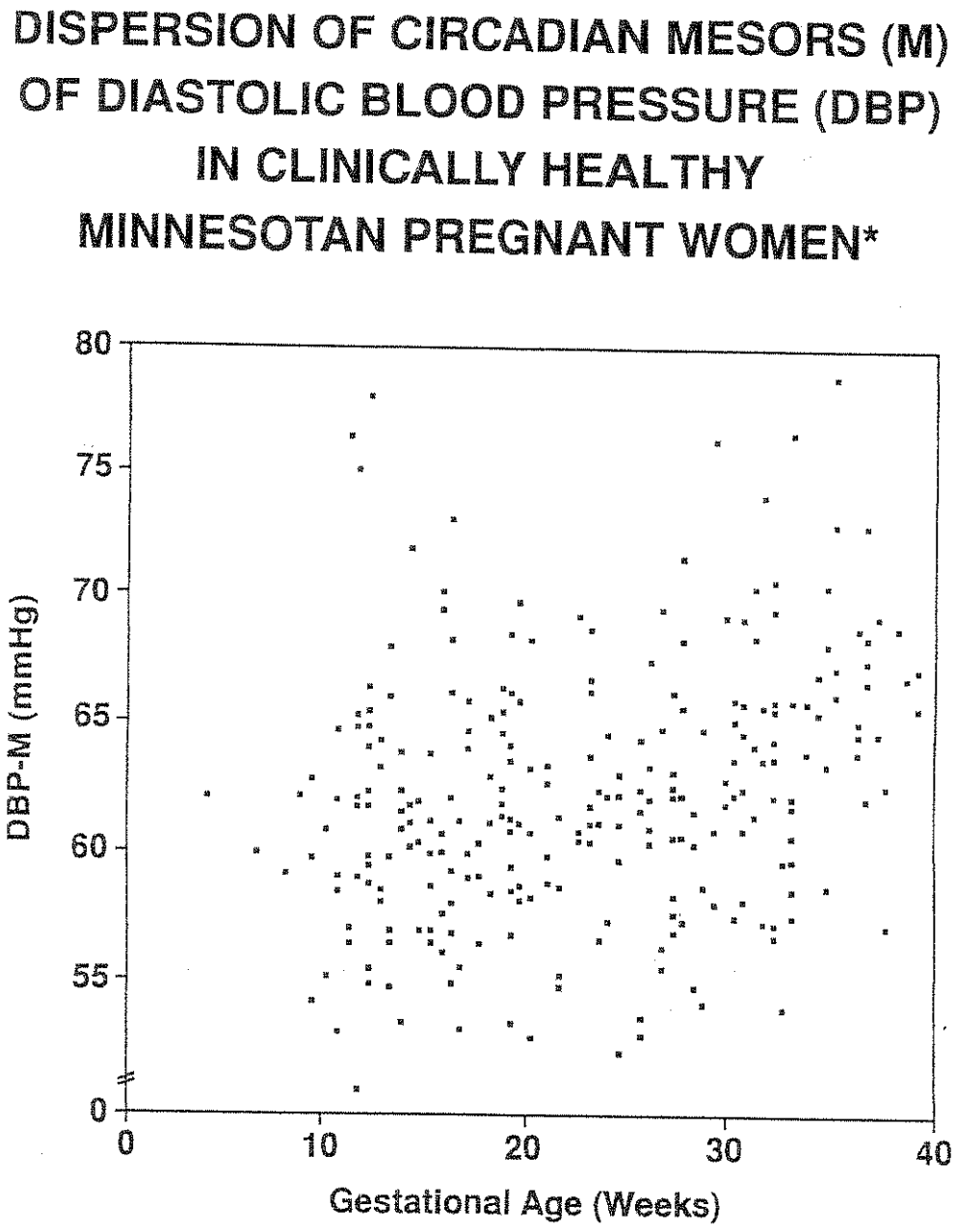
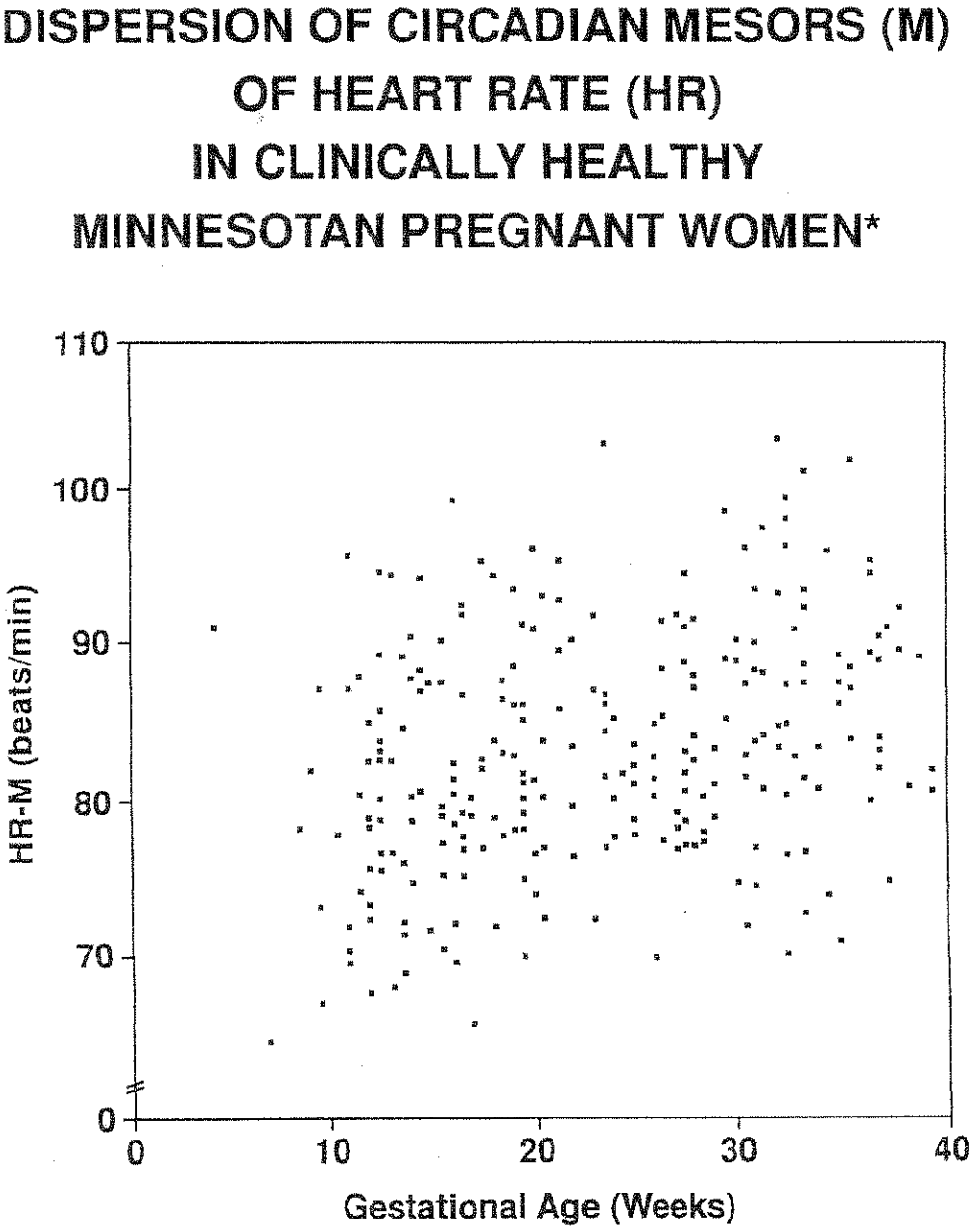


Figure 3. Transversely, across individuals, there is a large scatter in the circadian MESOR of heart rate. If ignored, this inter-individual variability obscures changes that take place during gestation.

C44



* Determined on the Basis of Ambulatory Monitoring (Colin Medical Instruments Corp., University Technology Park, 5850 Farinon Dr., San Antonio, TX 78249) at 1-Hour Intervals for 2 Days for a Total of 262 Profiles Provided by 161 Pregnant Women; Total Number of Measurements Exceeds 12,500

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Figure 4. Pairing across consecutive 48-hour profiles contributed by the same women in different trimesters reveals an initial decrease in blood pressure that reaches a minimum during the second trimester, and a steady increase in heart rate throughout gestation.

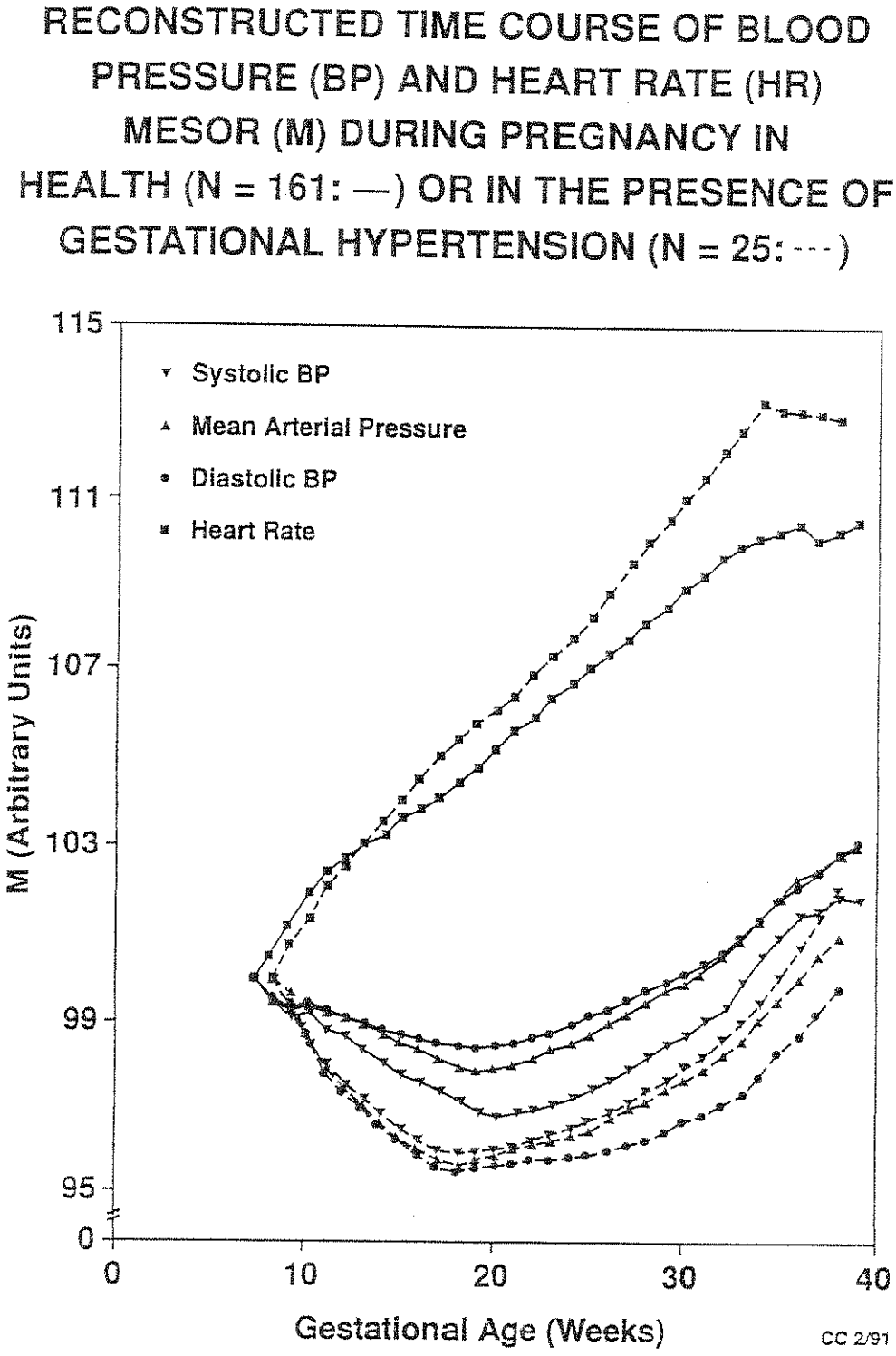


Figure 5. Differences in mean arterial pressure of the order of 8 mmHg are found well within the range of currently acceptable values between women who are clinically healthy at the outset but will develop gestational hypertension or pre-eclampsia and those whose pregnancy will remain uncomplicated.

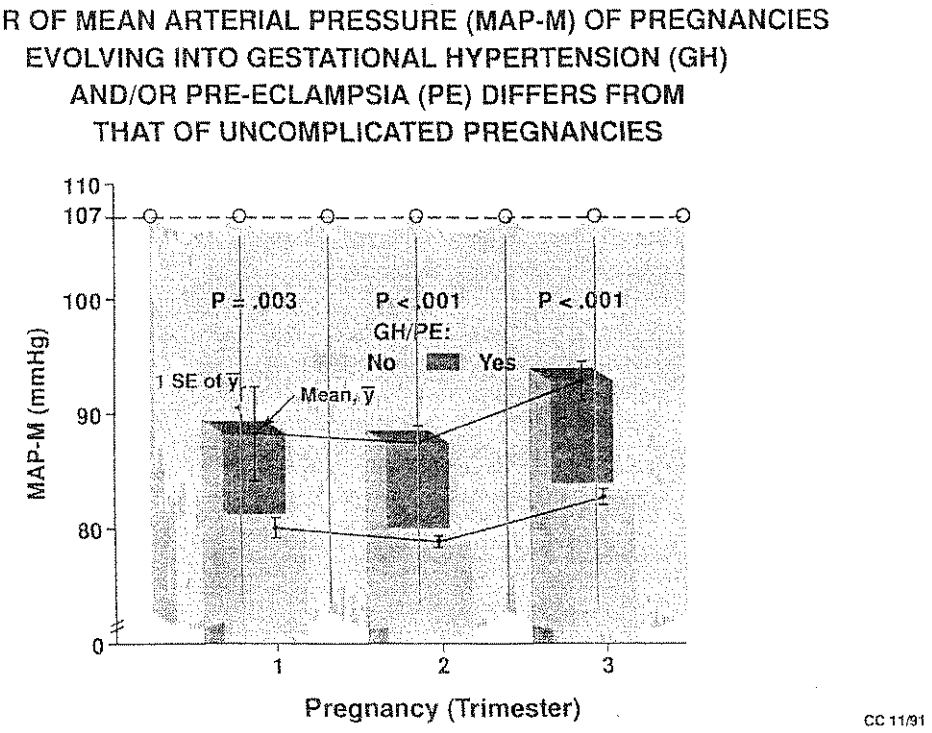


Figure 6. Pregnant women who are to develop gestational hypertension and/or pre-eclampsia later during pregnancy differ in terms of their circadian amplitude of blood pressure from women whose pregnancy will remain uncomplicated.

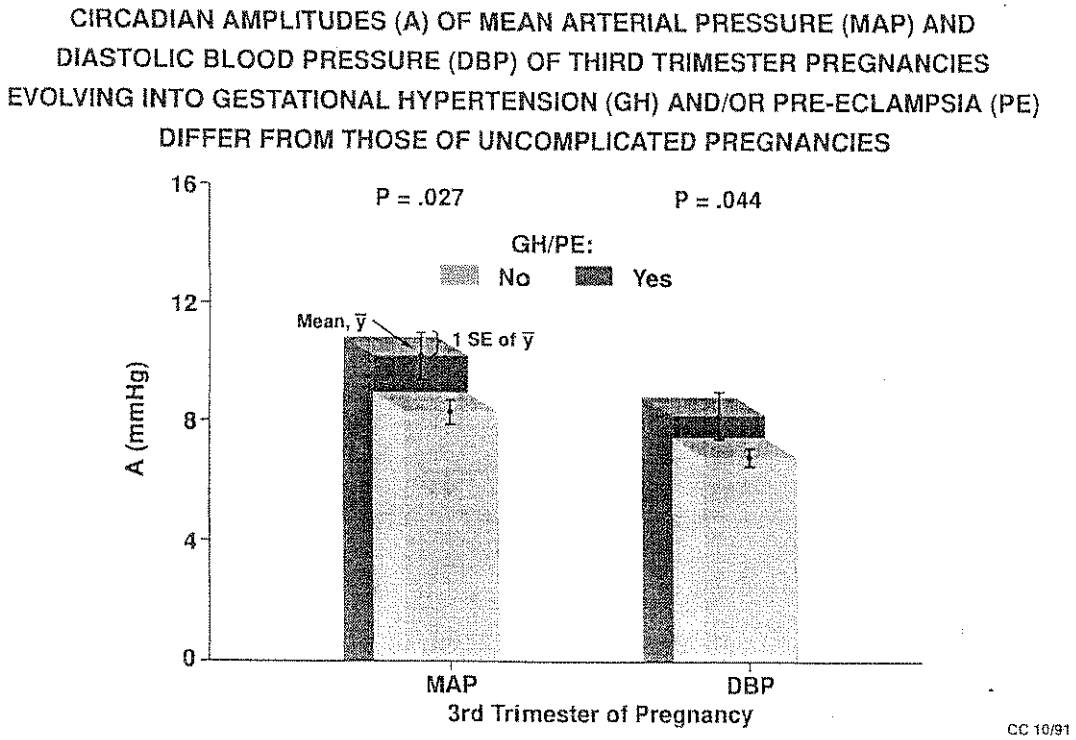
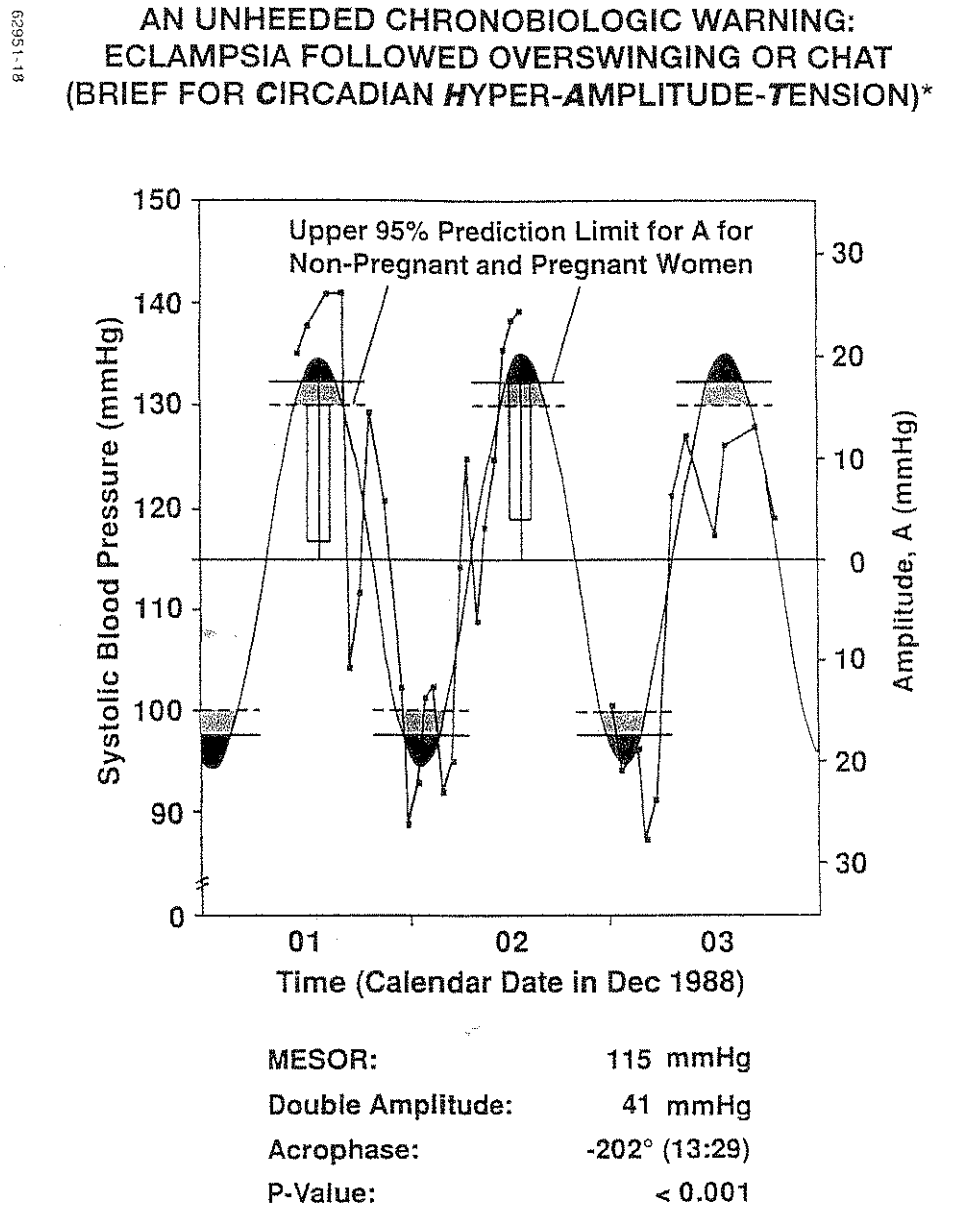


Figure 7. Blood pressure profile of pregnant woman. Around an acceptable rhythm-adjusted mean (MESOR), the extent of the circadian variation is found to be excessive by comparison with the circadian variation characterizing clinically healthy pregnant women or non-pregnant women of a similar age. Eight weeks after these data were collected, severe pre-eclampsia led to the delivery of a very premature boy who needed hospitalization for most of the first 26 months of his life.



* 8 weeks later, appearance of convulsions, delivery of boy in 27th gestational week, whose cost-accounted care during first 13 months totals U.S. $615,000; 26-month hospitalization may have raised cost to about U.S. $1 million.

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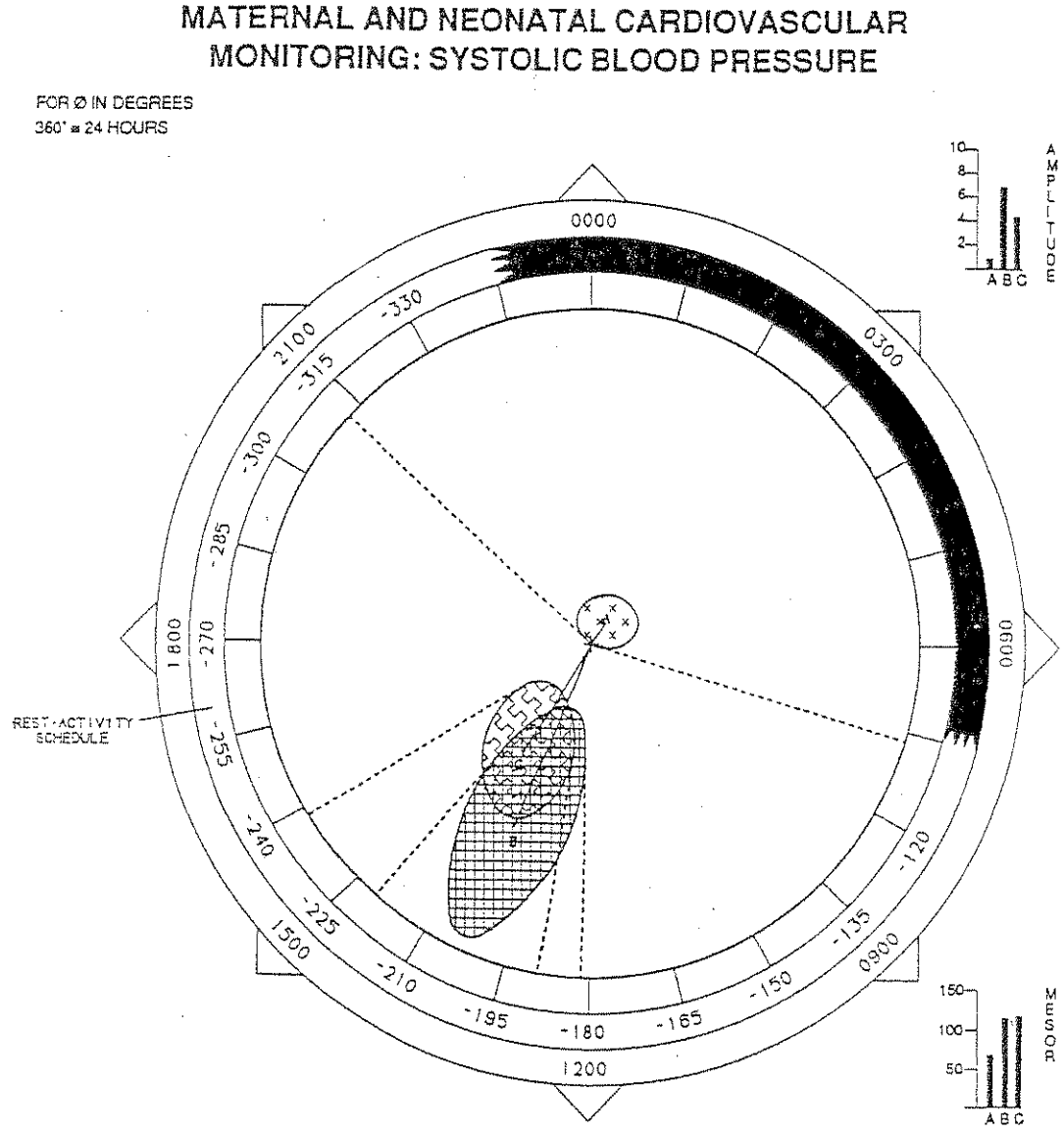
Figure 8. During the first week post-partum, a circadian rhythm of small amplitude is expressed for systolic blood pressure. It is in almost antiphase with the circadian rhythm of the maternal systolic blood pressure recorded before and after delivery.



KEY	P	NS	PR	MESOR +-SE	AMPLITUDE *	ACROPHASE (Ø) *
NEWBORNS: A	.043	25	6	68.36 1.61	0.98 (.03 1.94)	-35° (-312 -108)
MOTHERS:						
BEFORE DELIVERY: B	.007	8	21	117.48 2.58	6.7 (2.39 11.00)	-203° (-180 -220)
AFTER DELIVERY: C	<.001	23	16	122.50 2.35	4.2 (1.90 6.56)	-210° (-188 -237)

P = PROBABILITY OF HYPOTHESIS: AMPLITUDE = 0; N = NUMBER OF OBSERVATIONS
NS = NUMBER OF SERIES USED BY THE MEAN COSINOR TECHNIQUE TO FIND ELLIPSE
*CONSERVATIVE 95% CONFIDENCE LIMITS (PARENTHESES) DERIVED FROM COSINOR ELLIPSE

Figure 9. The circadian rhythm characteristics of systolic blood pressure are changing rapidly during the first few weeks of life. The acrophase moves toward the afternoon hours, and the amplitude increases.

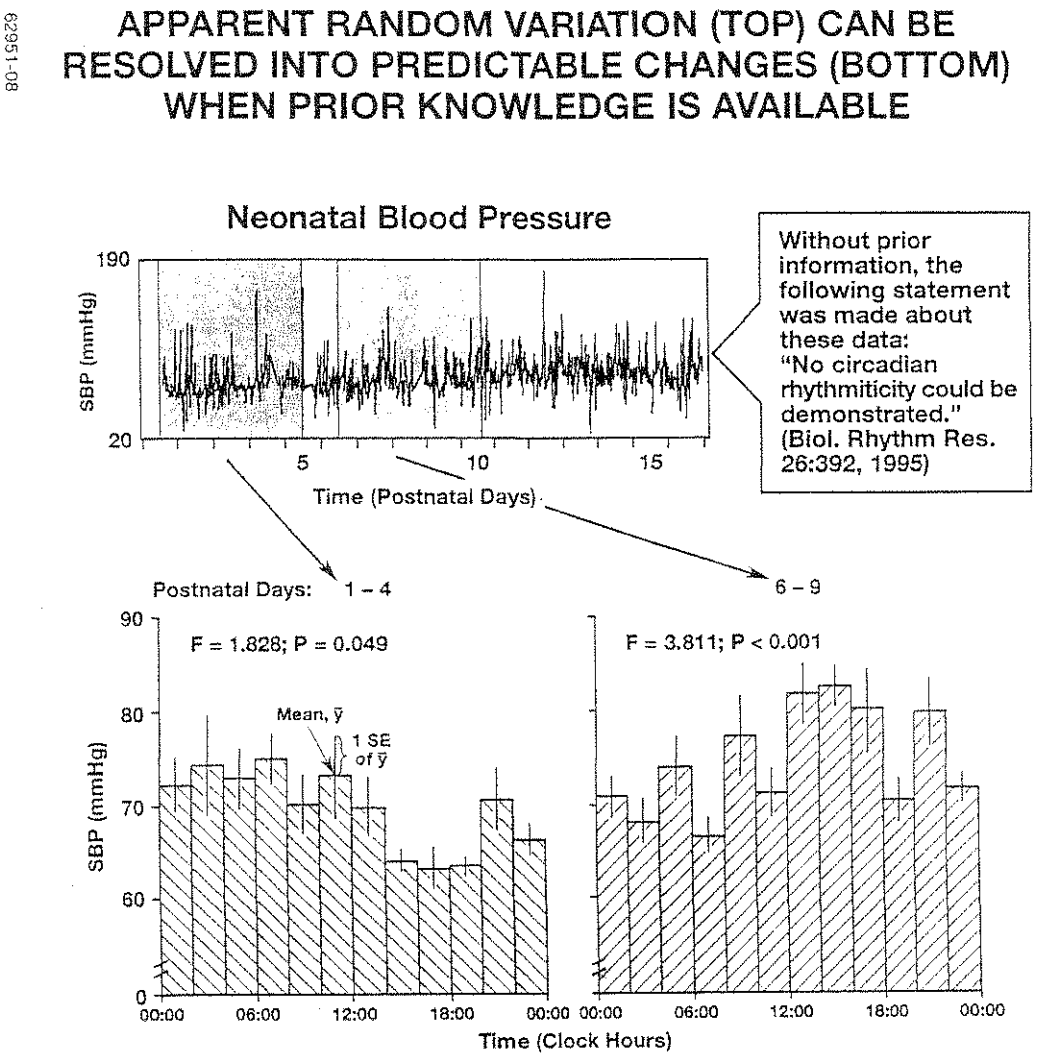
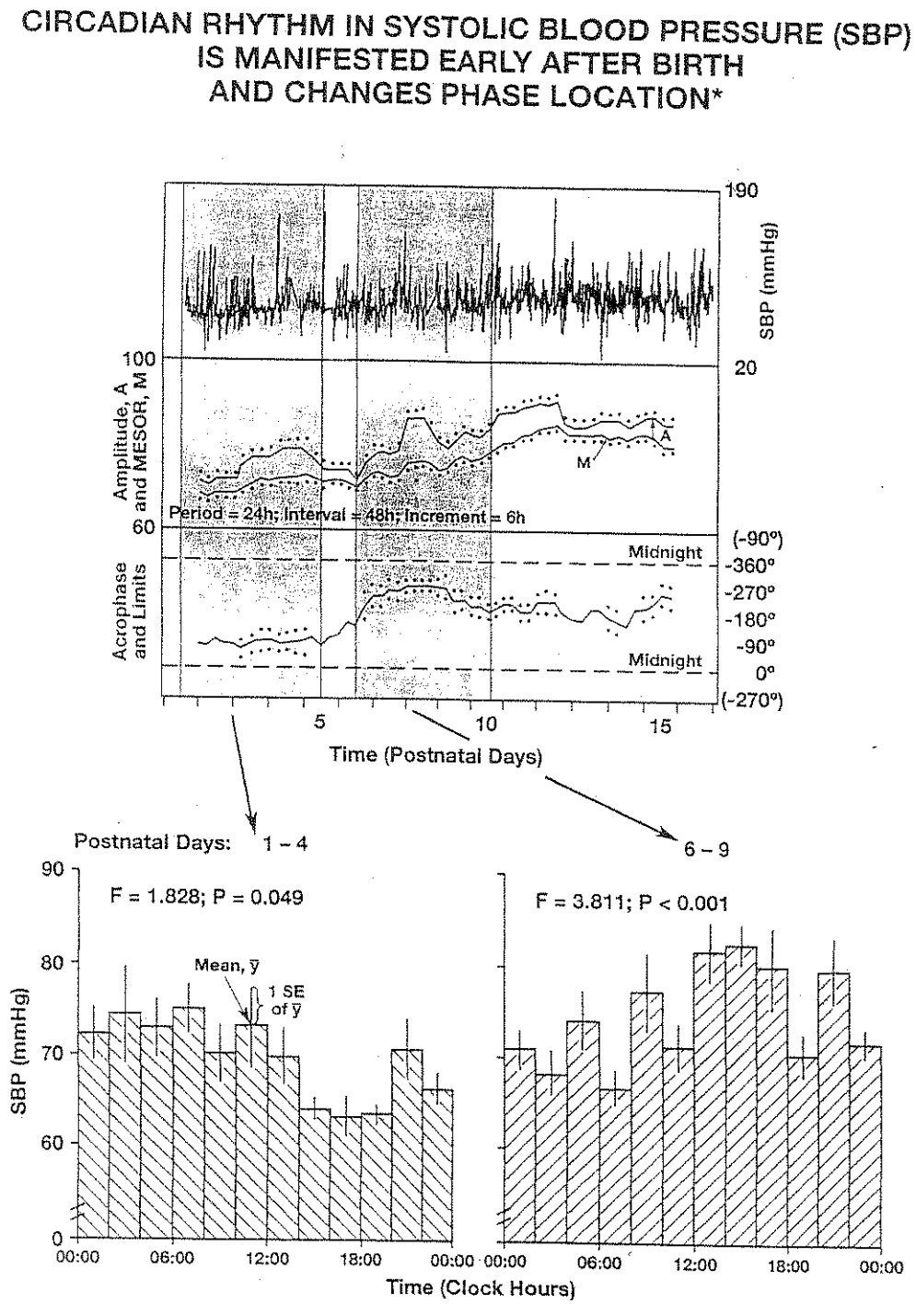


Figure 10. The adjustment of the circadian acrophase can be relatively abrupt in some cases, like in this clinically healthy baby. Between days 5 and 6, the acrophase changes from the early morning hours to the afternoon.



* Clinically healthy baby (Sp01).

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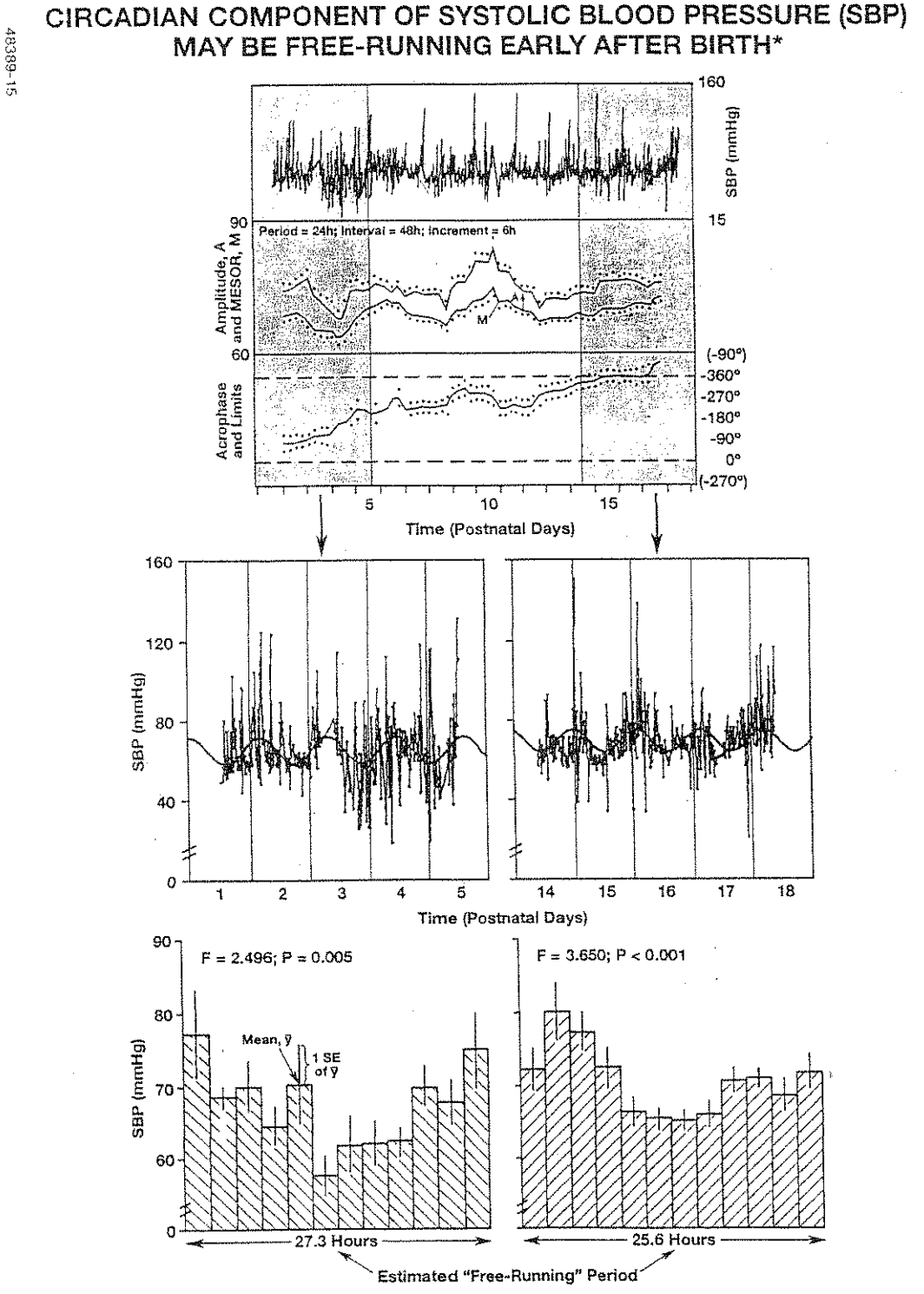
Figure 11. The adjustment of the circadian rhythm in systolic blood pressure can take longer than 2 weeks in some babies. A slow drift of the acrophase is reflected by a free-running circadian component that can assume different period lengths at different post-natal ages.



* Clinically healthy baby (Sp06), showing phase-drift spanning nearly a cycle, with period wobble.

CC 1/96

Figure 12. Exposure in utero to betamimetic drugs is associated with a larger circadian amplitude of systolic and diastolic blood pressure. Because a larger amplitude is often associated with a positive family history of high blood pressure and related diseases, this result can be interpreted as an undesirable drug effect, which may increase the vascular disease risk of the offspring.

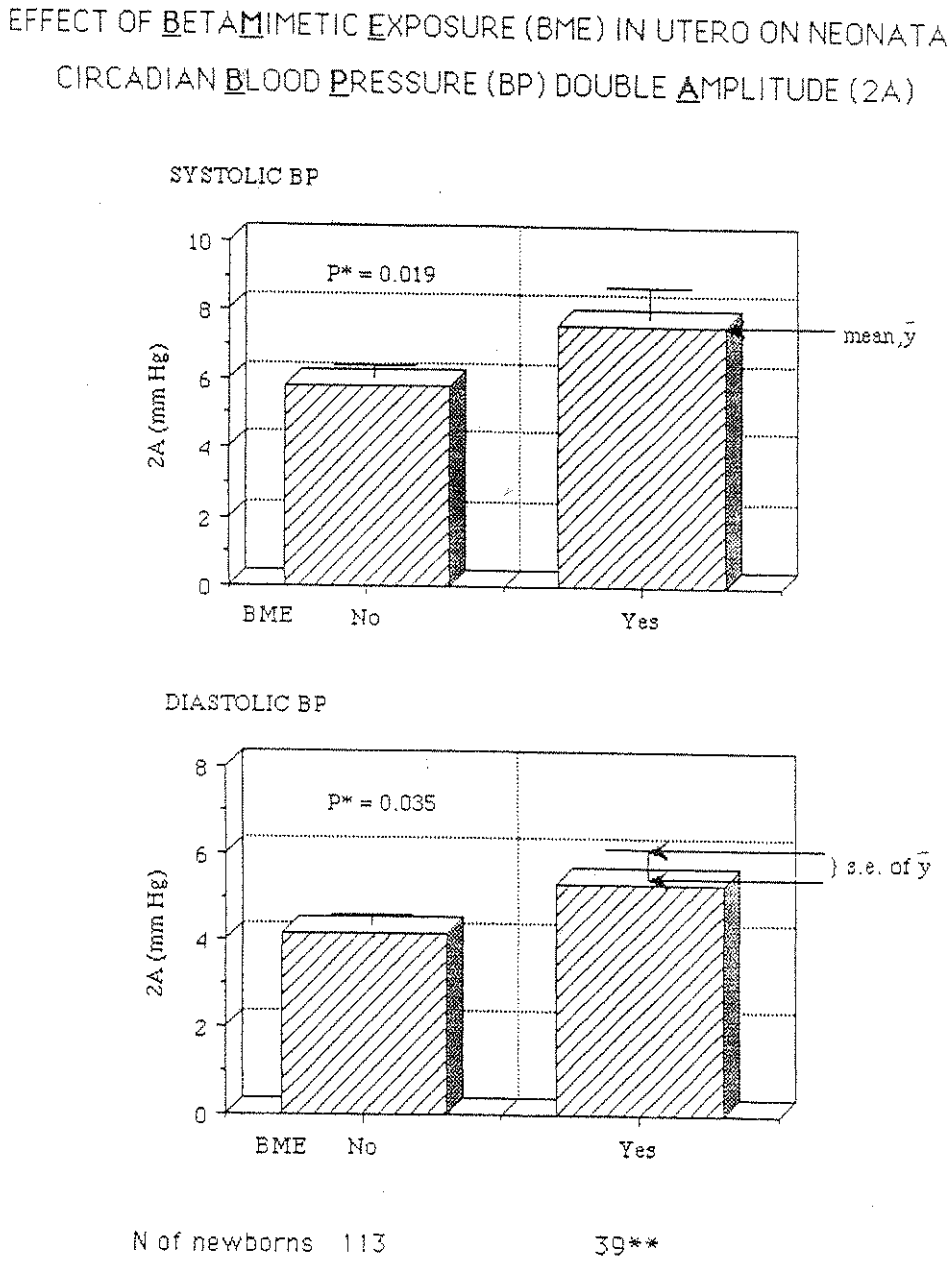
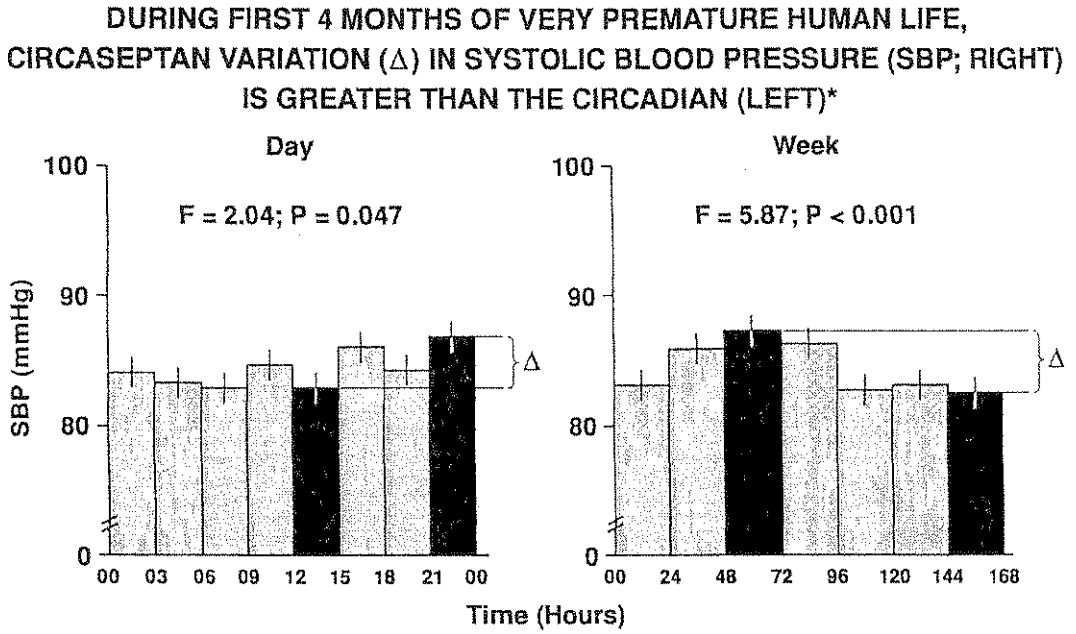


Figure 13. During the first 4 months of life of a very premature boy monitored in hospital, the about-weekly variation of his systolic blood pressure is more pronounced than the circadian variation, both components detected with statistical significance by one-way analysis of variance.



* Around-the-clock SBP, detrended by fit of 5th degree polynomial, was used for folding (stacking) into an idealized day (left) or week (right); deviation of endogenous periods from exact day or week likely reduce extent of predictable change within each cycle.

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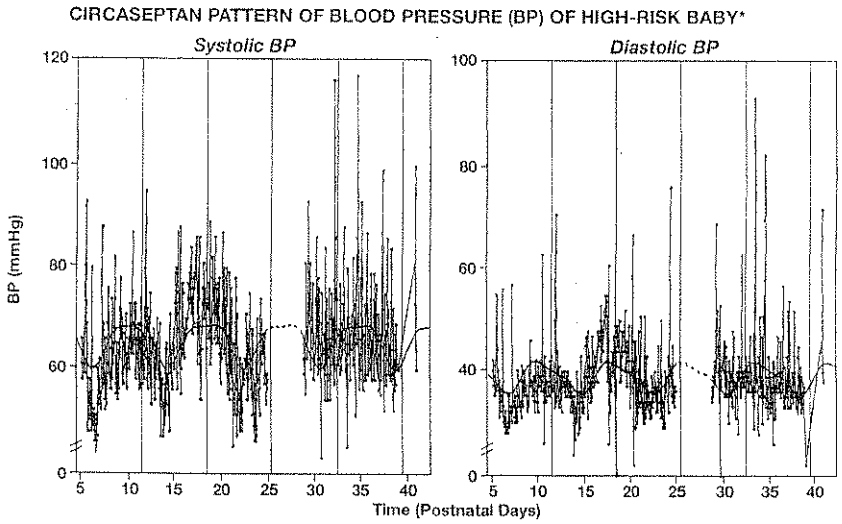
A10

Figure 14. The about weekly variation in systolic and diastolic blood pressure is visible to the naked eye in the case of a premature girl delivered at 31 weeks, with respiratory distress syndrome and on ventilation support. She died at 164 days, about 3 months after this record was obtained.



Nonlinear Results**	τ (Hours)	A (mmHg)	τ (Hours)	A (mmHg)
Circaseptan	195.0 (187.4; 203.9)	4.2 (2.4; 5.9)	190.3 (181.8; 199.4)	2.9 (1.6; 4.2)
Circasemiseptan	97.5	1.3 (0.0; 3.1)	95.1	0.8 (0.0; 2.1)

* S03 (F, born at 02:00 on 30 May 1992; BW = 1200 g; GA = 31 wks) monitored June 4 – July 10 on ventilation support with respiratory distress syndrome (died at 164 days).
** τ = period; A = amplitude; 95% confidence limits in ( ).

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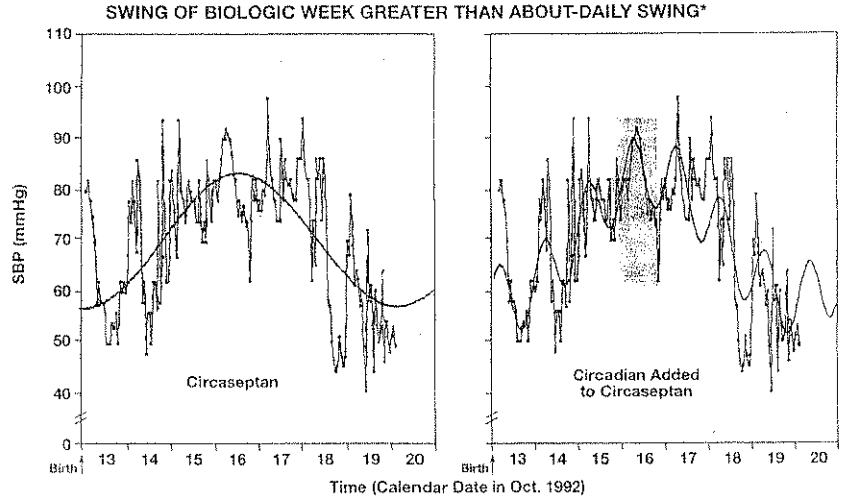
48188-11

Figure 15. Both the circadian and the circaseptan variations are assessed in the record of a premature girl born at 28 weeks. Both components have unusually large amplitudes. This baby, with several health problems, died about 5 days after this record was obtained.



Nonlinear Results**	τ (Hours)	2A (mmHg)	τ (Hours)	2A (mmHg)
	171.1 (145.7; 199.2)	26.4 (19.5; 33.3)	163.7 (136.6; 194.0)	25.3 (18.1; 32.6)
			24.6 (23.7; 25.6)	14.1 (6.8; 21.5)

* Predominance of circaseptan over circadian variation in systolic blood pressure (SBP) of high-risk infant: 14 znx (F; GA = 28 wks; BW = 1255 g) suffering from hyaline membrane disease, atelectasis, periventricular leucomalacia and subependymal hemorrhage, on ventilation support (CMV + PEEP), who died at 25 days of age.
** τ = period; 2A = double amplitude; given with 95% confidence limits in ( ).

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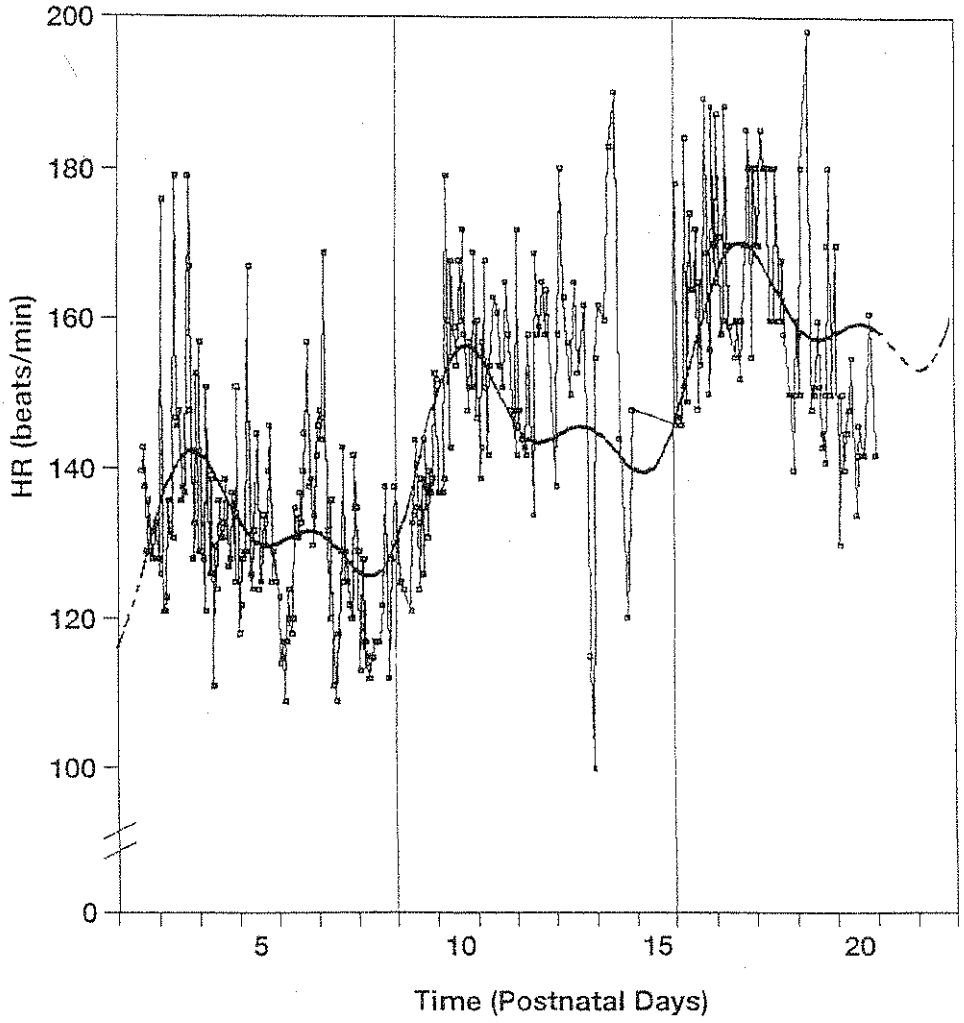
48386-10

Figure 16. Both an increase in heart rate during the first 3 weeks of life and an about 7-day component can be seen in the record of a baby boy born at 37 weeks.

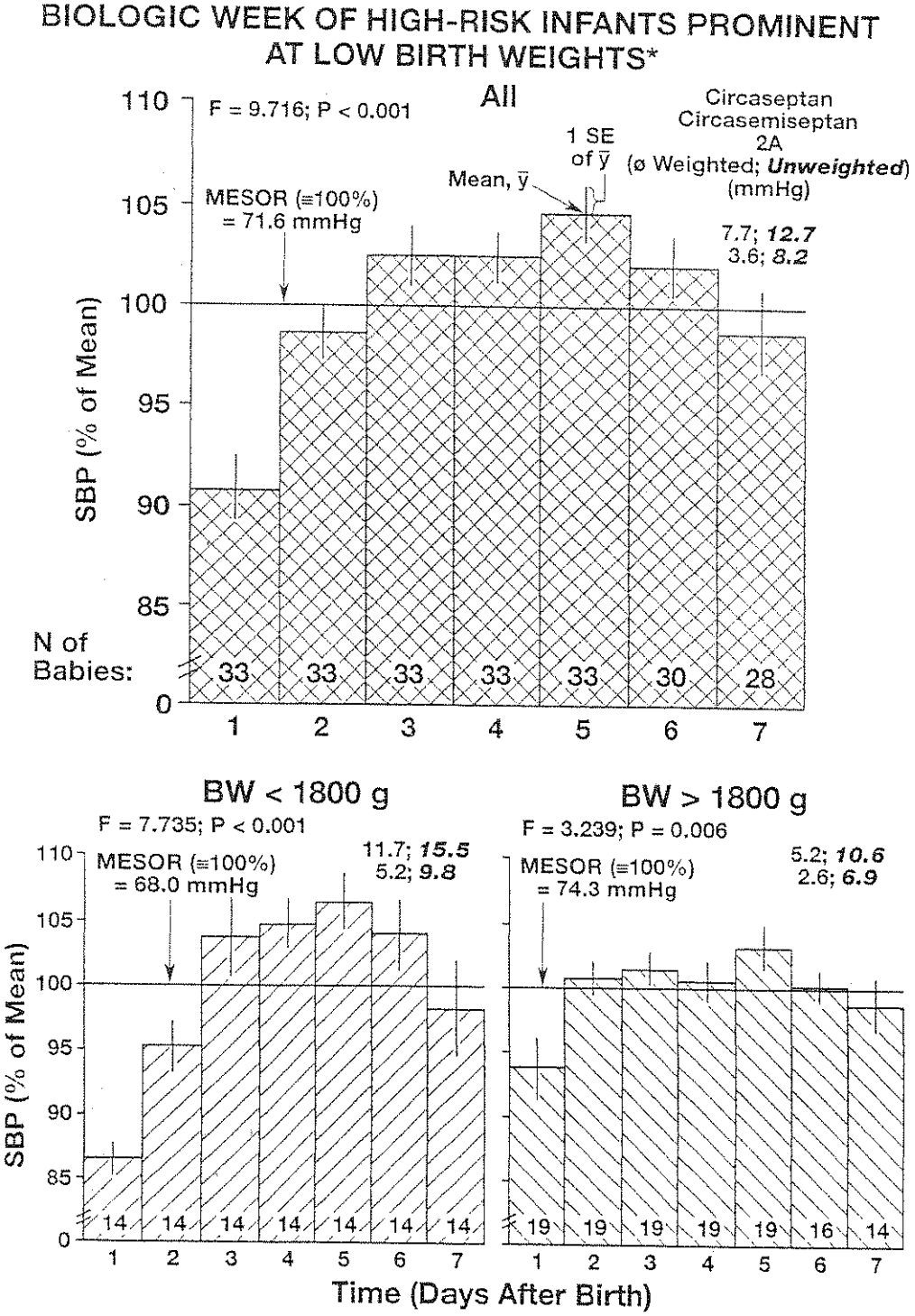


Nonlinear analysis reveals with linear trend, a circaseptan component with period (95% confidence limits) of 165.9 (157.4; 175.2) h and amplitude of 9.9 (6.0; 13.8) beats/min; model includes second harmonic with period 82.9 h and amplitude of 5.3 (1.3; 9.4) beats/min.

* S18 (M, born at 20:10 on 14 Aug 1990; BW = 2900 g; GA = 37 wks) monitored Aug 15 – Sep 3, 1990 on ventilation support suffering from intraventricular hemorrhage.

CC 1/96

Figure 17. On a group basis, the amplitude of the circaseptan variation is larger for babies with a birth weight less than 1800g than for baies with a birth weight above 1800 g. A low birthweight may thus be associated with an amplified circaseptan rhythm.



* About weekly (circaseptan) pattern of systolic blood pressure (SBP) is more prominent in babies with low birth weight (BW). Babies with a BW < 1800 g have larger 7- and 3.5-day double amplitudes (2A) (P < 0.05) and lower SBP MESOR (P = 0.008) than babies with a BW > 1800 g; ø = acrophase.

CC 1/96

Figure 18. Circaseptans can also be documented in healthy full-term babies.

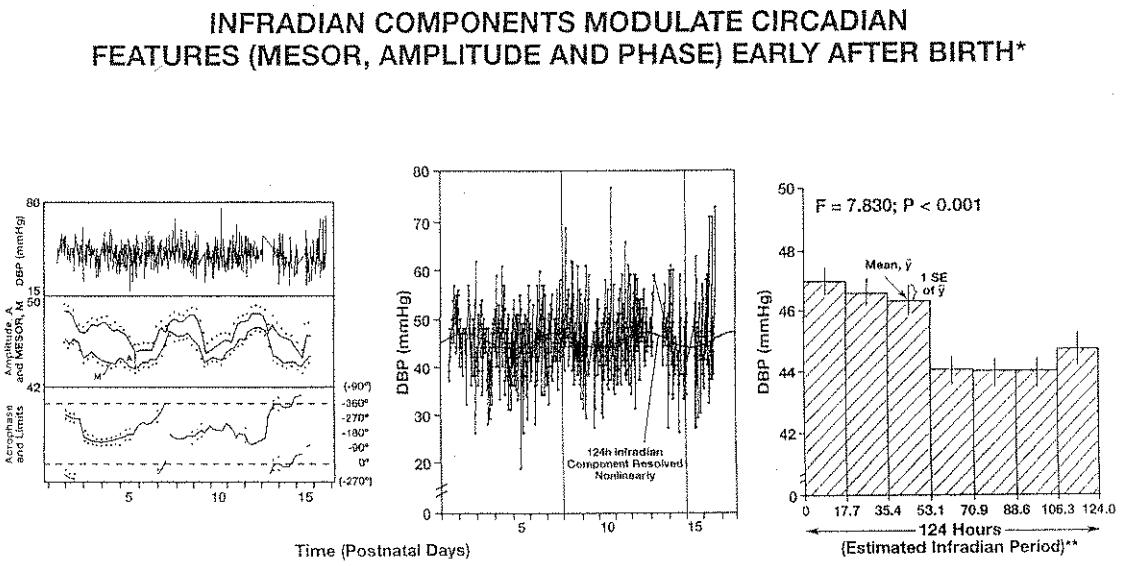


Figure 19. That the circaseptan component is partly inherited is suggested by the fact that the weekly pattern is more prominent and is statistically significant when it is assessed as a function of post-natal age (left), whereas it is weaker and not statistically validated by one-way ANOVA or by population-mean cosinor when the data are interpreted as a function of the day of the week (right).

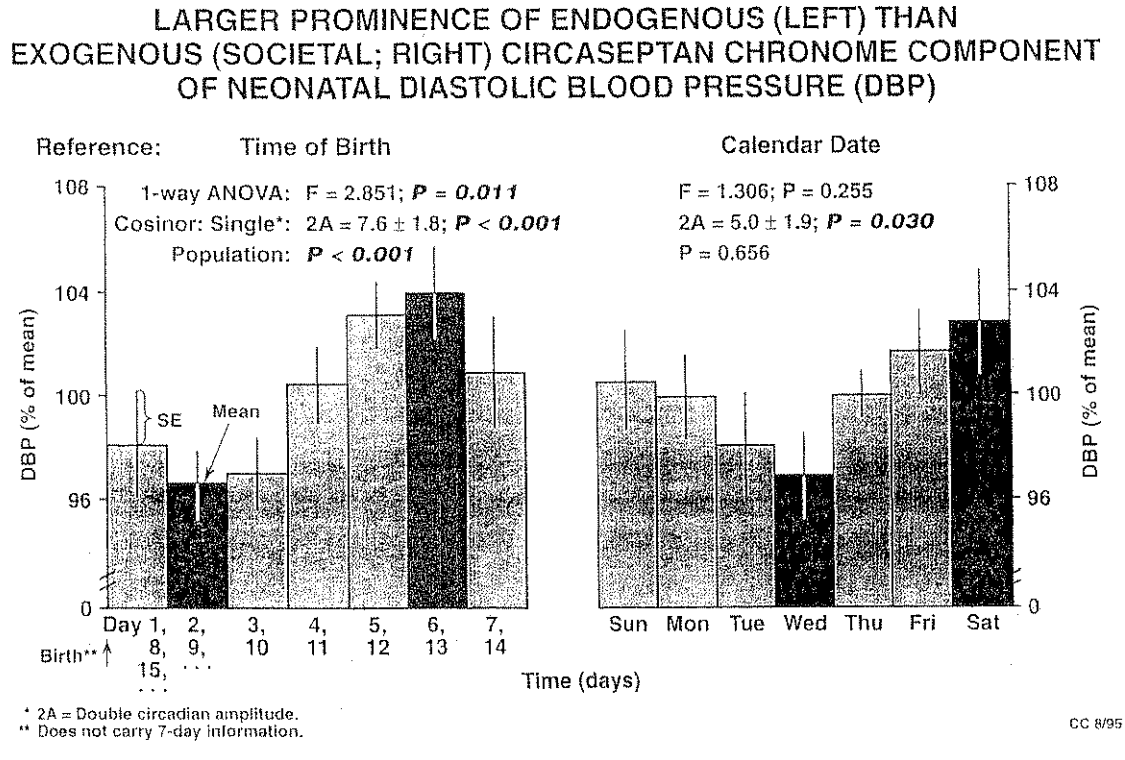
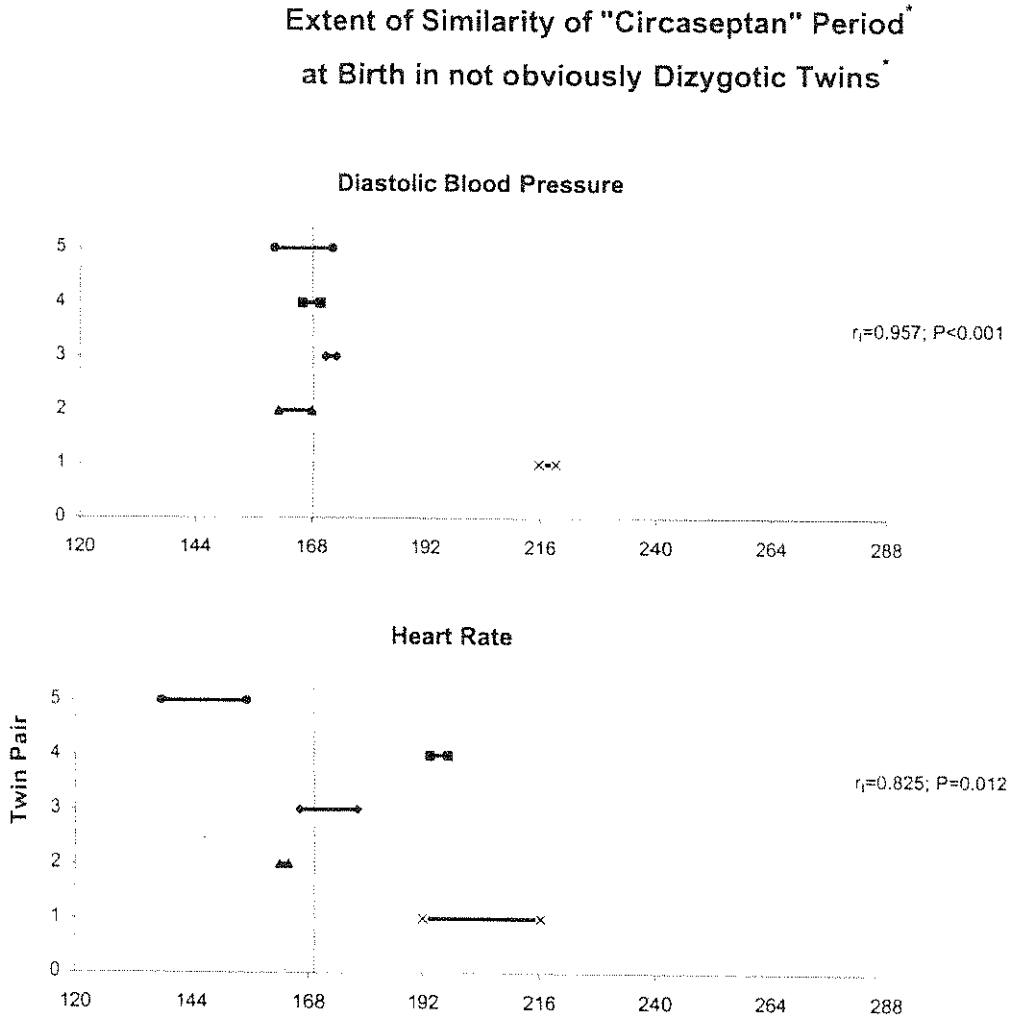


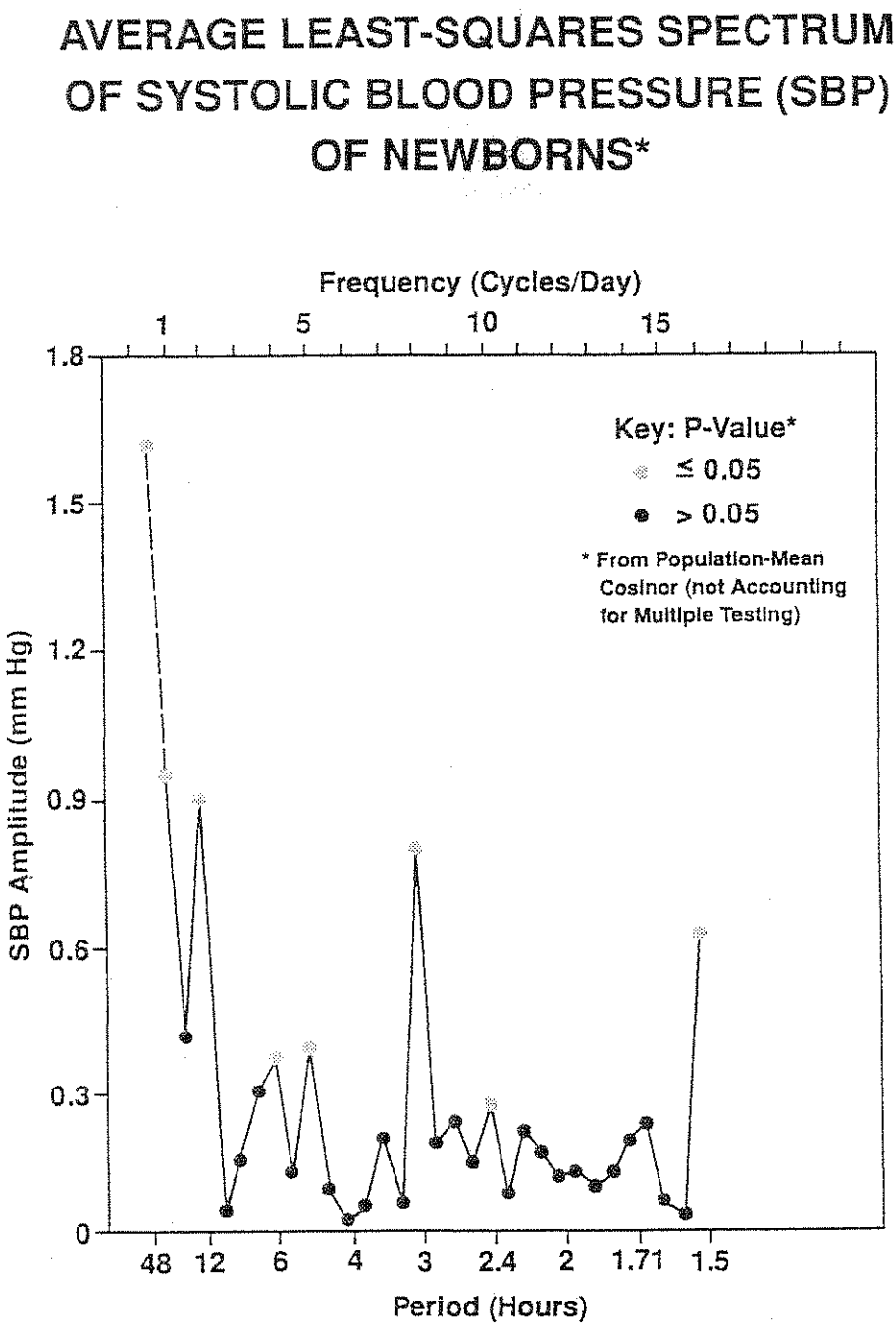
Figure 20. Endogenicity of the circaseptan component is suggested by a study on twins providing longitudinal records of blood pressure and heart rate in the neonatal intensive care unit. For each individual data series, the circaseptan period was determined by nonlinear least squares. An intra-class correlation coefficient comparing the intra- versus the inter- twin-pair variability finds twins within a pair more similar than twin pairs are among themselves.



*168 hours = 1 week; r_I: intra-class correlation coefficient; P:P-value testing H_0:r_I=0.

*Excluding twins of different gender and with dichorionic placenta

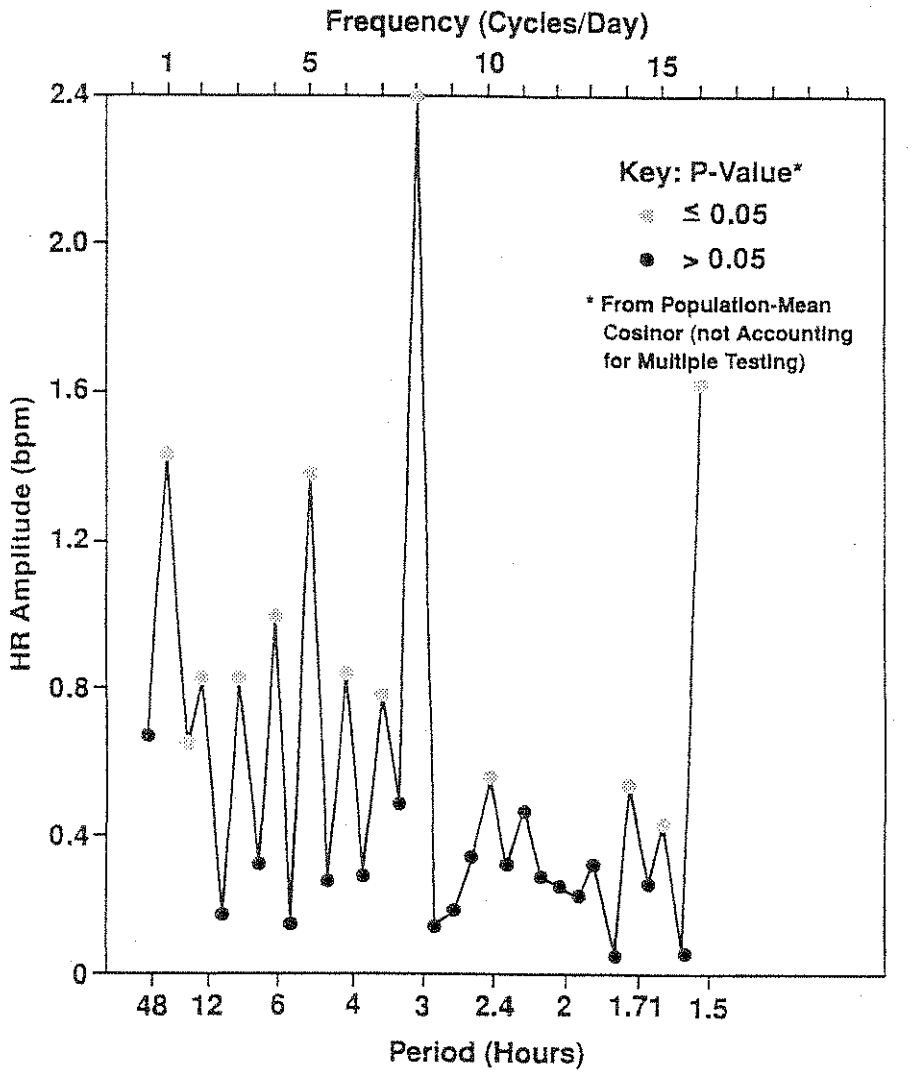
Figure 21. In addition to the circadian rhythm expressed at birth, a population mean-cosinor spectrum reveals the presence of a 12-hour harmonic of similar prominence than the circadian component. Ultradians are also detected, notably an about 3-hour component that is likely related to the feeding schedule.



* 164 Newborns Contributed a 48-h Profile (at 30-min Intervals) During the First Week of Life, Using an Automatic Monitor from Colin Medical Instruments (12677 Silicon Drive, San Antonio, TX 78249)

CC 11/89

Figure 22. The 3-hourly component is particularly prominent in the population-mean cosinor spectrum of heart rate.



* 164 Newborns Contributed a 48-h Profile (at 30-min Intervals) During the First Week of Life, Using an Automatic Monitor from Colin Medical Instruments (12677 Silicon Drive, San Antonio, TX 78249)

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Table 1: Circadian rhythm in blood pressure and heart rate of clinically healthy newborns*

Variable	Percent Rhythm (%)	P-value	MESOR	Circadian double Amplitude	Acrophase (360° = 24h)
All (N=164)					
SBP (mmHg)	6	<0.001	71.8	1.88	-97°
DBP (mmHg)	7	<0.001	44.1	1.32	-90°
HR (bpm)	4	<0.001	124.9	2.80	-26°
NFH (N=70)					
SBP	6	0.103	71.7	1.42	-91°
DBP	7	0.091	44.0	1.02	-83°
HR	4	0.012	126.2	2.08	-18°
PFH (N=94)					
SBP	6	<0.001	71.8	2.22	-100°
DBP	7	0.001	44.1	1.54	-94°
HR	4	<0.001	124.0	3.34	-29°

*Monitored automatically for 48 hours at 30-min intervals during the first week of life.
SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; HR: Heart Rate.
NFH and PFH: newborns with a negative (NFH) or positive (PFH) family history of high blood pressure and/or related cardiovascular diseases.

Table 2: Circadian component of blood pressure of clinically healthy full-term neonates during the first 2 days post-partum*

Variable (mmHg)	Percent Rhythm (%)	P-value	MESOR ± SE (mmHg)	Circadian double Amplitude (95% CI)	Circadian Acrophase (360°=24h) (95%CI)
SBP	4.3	0.009	66.9 ± 0.8	2.3 (0.9; 3.8)	-98° (-60; -136)
DBP	3.6	0.016	40.8 ± 0.5	1.6 (0.5; 2.6)	-100° (-64; -146)

*SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure.

LONG-TERM, WHEN-NEEDED LIFELONG MONITORING CONCERNS GOVERNMENTS, ETHICS COMMITTEES AND EVERYBODY*

Othild Schwartzkopff*, Germaine Cornélissen*, Christopher Bingham*, Pavel Homolka•, George Katinas*, Robert P. Sonkowsky*, Franz Halberg* and the International BIOCOS Project Team§

*University of Minnesota, Minneapolis, MN, USA
oMasaryk University, Brno, Czech Republic

Address correspondence to:

Othild Schwarzkopff, M.D.
Assistant professor of pediatrics emerita
Halberg Chronobiology Center,
University of Minnesota
715 Mayo Building
Mayo Mail Code 8609
420 Delaware St. S.E.
Minneapolis, MN 55455, USA

*Invited lecture on chronomics at Masaryk University, Brno, Czech Republic

Key words: chronomics, chronobiology, history of science, history of statistics, time series, Wolf's relative sunspot numbers

§Armenia: Noubar Aslanian, Institute of Cardiology, Yerevan.
Austria: Manfred Herold, University of Innsbruck.
Belgium: Christian Muller, Olga Quadens, Aerospace Institute, Brussels; Alain Delcourt and Guy Toussaint, private practice physicians, Brussels.
Brazil: Ana Amélia Benedito Silva, Nelson Marques, Luiz Menna Barreto, University of Sao Paulo.
Bulgaria: Nadejda Madjirova, Department of Psychiatry, Medical University, Plovdiv.
China: Zhengrong Wang, West China University of Medical Sciences, Chengdu; Ziyan Zhao, Shandong Academy of Medical Sciences, Jinan.
Czech Republic: Bohumil Fiser, Jarmila Siegelova, Jiri Dusek, Pavel Homolka, Muhammad Al-Kubati, Pavel Prikryl, Masaryk University, Brno.
Germany: Sigrid Berger, Max-Planck-Institute für Zellbiologie, Ladenburg; Armin Grafe, GFZ Potsdam, Niemegk; Karl Hecht, Charité Hospital, Humboldt University, Berlin; Max Halhuber, Bad Berleburg; Thomas Müller-Bohn, Pharm.D., Süsel-Holstein.
India: R.B. Singh, Medical Hospital and Research Centre, Centre for Nutrition and Heart Research, Moradabad; R.K. Singh, Department of Biochemistry, King George's Medical College, Lucknow.
Italy: Franca Carandente, Department of Chronobiology, and Cristina Maggioni, Department of Obstetrics, University of Milan; Federico Perfetto, Roberto Tarquini, Riccardo Livi, Department of Medicine; Giorgio Mello, Department of Obstetrics, and Giancarlo Mainardi, Department of Pediatrics, University of Florence; Andrea Galvagno, Maurizio Montalbini, Pioneer Researches, Ancona.
Japan: Kuniaki Otsuka, Yoshihiko Watanabe, Department of Medicine, Tokyo Women's Medical University, Tokyo; Terukazu Kawasaki and Keiko Uezono, Institute of Health Sciences, Kyushu University, Fukuoka; Yuji Kumagai, Jichi Medical School, Tochigi.
Mexico: Salvador Sánchez de la Pena, Instituto Mexicano del Seguro Social, Mexico City; Clicerio Gonzalez, Centro de Diabetes Mexico City; Maria Luisa Fanjul Moles, L. Neurofisiologia Comparada, Coyoacan.
Nigeria: S.O. Ogunade, Obafemi Awolowo University, Ile-Ife.
Norway: Andi Weydahl, Finnmark College, Alta.
Romania: Ion Baciu, Professor and Head of Physiology, Oradea University, Oradea.
Russia: Mikhail Blank and Olga Denisova, N.N. Petrov Oncology Research Institute, Ministry of Health, and Mikhail Denisov, St. Petersburg Special Mental Hospital, St. Petersburg; Tamara K. Breus, Space Research Institute, Moscow; Maria Mitish, Elena V. Syutkina, Tatiana Turti, Galina V. Yatsyk, Institute of Pediatrics, Academy of Medical Sciences, Moscow; Anatoly Masalov, Lebedev Physics Institute, Moscow; Rina M. Zaslavskaya and Mikhail M. Teibloom, Moscow Hospital #60, Moscow.
Slovakia: Miroslav Mikulecky Sr., Comenius University, Bratislava; Alexander Kreze, Lubochna.
South Africa: Mary-Jane Thaela, Animal Nutrition and Animal Products Institute, Irene.
Spain: Leopoldo Garcia Alonso, Hospital Materno-Infantil 'Teresa Herrera', La Coruna; Francisco del Pozo Guerrero, Maria Teresa Arredondo Waldmeyer and

Maria José Rodriguez Fernandez, Department of Telecommunications, Universidad Politecnico de Madrid; Miguel Revilla, Department of Mathematics and Computer Science, University of Valladolid.
Sweden: Lennart Wetterberg, Karolinska Institute, Stockholm.
Ukraine: Anatoly Delyukov, Senior Scientist, Institute of Physics, Ukrainian Academy of Sciences, Kiev; Yuri Gorgo, Department of Human and Animal Physiology, Faculty of Biology, Kiev National Taras Shevchenko University, Kiev.
UK: Douglas Wilson, ADAS Bioengineering Unit, Cheltenham, England; Sam K. Arthur, Gastroenterology Department, Queen Mary and Westfield College, University of London, England; Hugh Simpson, Department of Surgery, University of Glasgow, Scotland.
USA: Germaine Cornélissen, Franz Halberg, Julia Halberg, Chronobiology Laboratories; Dana Johnson, Department of Pediatrics; Christopher Bingham, Douglas Hawkins, Department of Applied Statistics; Jim Holte, Department of Electrical Engineering; Robert Patterson, Bakken Medical Instrumentation and Device Laboratory, University of Minnesota, Minneapolis, Minnesota; Mark Engebretson, Department of Space Physics, Augsburg College, Minneapolis, Minnesota; Henry Fink, Richard Kopher and Joseph Rigatuso, HealthPartners, St. Paul, Minnesota; Earl E. Bakken, North Hawaii Community Hospital, Kamuela, Hawaii; Patrick Delmore, Medtronic Inc., Minneapolis, Minnesota; Francine Halberg, Marin General Hospital, Greenbrae, California.

ABSTRACT

A long history of disputed claims in astronomy, broader physics, modeling and forecasting statistics and many other fields, including economics, military science and biomedicine, prompts us here to revisit the initial progress, centuries ago, of knowledge from observations on the sun itself. Thereby, we wish to apotheosize Samuel Heinrich Schwabe, the discoverer of the sunspot cycle, into a pantheon of transdisciplinary science -- of chronomics, that is of the mapping of chronomes, time structures around us as well as in us -- and thus also into the pantheon of the mother science, chronobiology, concerned with intra-organismic and intra-societal mechanisms of time structures first and foremost. We also wish to set Schwabe apart from Wolf and Wolfer, who systematized and extended the scope what Schwabe brought cautiously to the fore. We consider sampling on associations on earth of cyclic solar activity. We dedicate this article to Jarmila Siegelova on her 60th birthday. Like Schwabe, she has started a systematic mapping, if not of sunspots, then of their signatures in everyday physiology along the scale of a week in newborns and adults. We trust that she and generations to follow will continue *ad multos annos*.

INTRODUCTION

Mapping time structure, chronomes, the field of chronomics, is new and has revealed the occurrence of infra-annual cycles with periods longer than a year (frequencies lower than 1 cycle/year, hence *infra*) (1-9). Any one multidecadal cycle can coexist with other spectral components, including some with an integral multiple or submultiple decadal frequency (9). Infra-annuals share with certain rhythms of shorter periods, like circadians and circaseptans, a critical importance and apparent ubiquity. Accordingly, and in keeping with some now-documented non-overlapping 95% confidence intervals between the periods of infra-annuals in the biosphere and in the environment (9-11), some if not all infra-annual rhythms may be in part genetically coded. Their study may repeat the story of circadians and circannuals, with obvious visible photic counterparts. But for one case among the many near-matches of the non-photic, not consciously sensed physical environmental cycles, such as the geomagnetic week, heritability can be suggested by studies of biological circaseptans: twin studies demonstrate a congenital basis. The possibility of heritability seems especially important for the interpretation of infra-annuals. With their putatively built-in mechanisms, infra-annuals can be subject to a drastic phase-dependence of responses to environmental effects. These seemingly paradoxical effects, the feedsidewards, were documented in vitro for circadians (12, 13); they lead in several cases to a sequence of stimulation, no-effect and inhibition by one entity upon another or several others (6).

Without considering chronomics (which includes, with the mapping of a spectrum of multifrequency cycles, chaos and trends, also an analysis of their genetically anchored feedsideward modulations), Chizhevsky suggested and descriptively documented that at least circadecadals contribute to epidemics of bacteriological contagion as well as to "contagions" of disturbed minds, such as political upheavals and wars (14-17). These topics benefited further from the scholarship of Suitbert Ertel (18-21) who elaborated on both kinds of contagion with new data, as does Miroslav Mikulecky (22-25). They have the courage of

undertaking a hazardous journey, since vastly different, sometimes discrepant effects become apparent in the context of a much disputed heliobiology (26, 27).

Prior claims of the effects of the sun, the cosmic rays they displace, and/or of geomagnetics, more often than not, were based on merely descriptive statistical evidence; sometimes opposite results were usually published without assessing the stage of infra-annual rhythms. Controversies become predictable when, e.g., feedsidewards can be shown by virtue of the rhythmically sequential and to that extent predictable insofar as rhythmically recurrent mapping of opposite or at least quantitatively very different associations. The mapping of these rhythms promises to resolve many controversies in a vast field described as secularity; it will require re-analysis, whether one deals with only one or with many prior investigations. Many numerical "responses" will not be resolved by current "meta-analyses" that do not inquire into the possibility that a phase-drifted or phase-shifted rhythm may be compared with a synchronized one, Figure 1. The many meaningless responses shown in this figure apply equally to sampling, on any rhythms and include studies that ignore circadecadals that can be great confounders (5).

On the positive side, the multidecadals may lead us to external or internal mechanisms that may allow us to objectively address some of the ills of society as well as those of individuals. With this view of preventive health care, blood pressure and heart rate chronomics are now investigated in different geographic locations, notably in St. Anna Hospital in Brno, thanks to Jarmila Siegelova and Pavel Homolka. The issue is whether this can be done as a public service, as clean and safe streets and a safe circulation should be (28), or whether monitoring has to be privatized, if people are not willing to adopt a procedure because it is free.

The sun and the earth are both magnets

Two transdisciplinary biologists have carved themselves a niche in a hall of fame of chronomics (the mapping of time structures, chronomes) (8): the physician William Gilbert (1544-1603) (29), and the pharmacist Samuel Heinrich Schwabe (1789-1875) (30-34; cf. 31, 35, 36), Figure 2. Gilbert, who was prominent in his profession as physician to Queen Elizabeth I and King James I of England, is recognized by physicists for publishing the first truly scientific treatise, "De Magnete", which apotheosizes in a transdisciplinary pantheon. Sydney Chapman and Julius Bartels, the leading geophysicists of their time, built on his statement that "the earth is a great magnet" (37). Schwabe discovered a cycle in the spots of an even greater magnet, the sun. Against the background of Gilbert's life, we focus on the discovery of sunspots, since they also exhibit a 50-year cycle corresponding numerically, at least to what Fiser et al. describe for the incidence of stroke in the Czech Republic, complementing a quindecadal cycle also found in Minnesota (6, 38). We herein explore the roots of a necessary and broad cartography of infra-annual rhythms.

The introduction of Schwabe into the pantheon of transdisciplinary science

We use the example of Schwabe's meticulous observations to indicate:

• First, that some correct, but cautious conclusions were drawn with relatively short time series, contrary to any fixed rule requiring a specifiable number of cycles (39), in keeping with an unpublished manuscript by Christian Horrebrow (1718-1776), who wrote in 1776 "... it is hoped that by means of frequent

observation, one will also find a period here similar to the movement of other heavenly bodies" (36);

- Second, that the data gathered centuries ago for a very few, if not one or two cycles, can be validated insofar as the occurrence of a cycle as such is concerned, in the light of data now covering centuries, although the particular initial span may not happen to be representative of an average based on the now-available series as a whole;
- Third, that an outcome on the surface of the earth of the sunspot cycle as a 10-year trade cycle (40) was postulated before Schwabe dared to write explicitly about periodicity;
- Fourth, that associations between the sunspot cycle and geomagnetics, based on the hint of similar cycle lengths found by Lamont (41), Wolf (42), Gautier (43) and Sabine (44) and associated by Johnson (45) also with Arago, who had followed terrestrial magnetism for over a decade from the 1820s to the early 1830s (45; cf. 46), are now known concerns, as magnetic storm effects upon the grids of power stations (47) and:
- Fifth, that multidecadal cycles, also affect the figurative grids of human growth (6), the brain (4) and the heart, as myocardial infarctions (6, 48, 49) that are not trivial; and
- Sixth, we point to the truism that the data on circadecadal or circamultidecadal cycles are not as readily replicated as circadians or even circannuals, and certainly not as readily as the ~10 Hz or 1 Hz of the human EEG or ECG or the alternating current at 50 or 60 Hz of any appliance. Hence, special comment on sampling requirements is mandatory, the more so since we are dealing with critical reference values for health and disease.

Lexicography

Two encyclopedias (50, 51) erroneously cite a report by Schwabe on the sun in 1843 (31) as the source for the discovery of an about-10-year cycle in the number of sunspots. In Schwabe's paper published in 1843 (31), the author referred, parenthetically, to a publication in 1838 (30), but in this publication there is no mention of any periodicity. The earliest record known to us, albeit without any reference to a cycle, is Schwabe's tabulation, in his 1838 publication, of 12 consecutive yearly values that speak for themselves (30). The tabulated number of sunspot groups he had observed each year between 1826 and 1837 does not require any reference to a cycle: the original data, in the published table, are so quasi-sinusoidally different from each other that they suggest a cycle to the naked eye, which, in the footsteps of innumerable others, we have readily confirmed by the result of a linear-nonlinear rhythmometry-based (52, 53) re-analysis, Figure 3. This procedure, in a first linear step, involving the fit of different fixed periods, identifies a cycle with a period of 8.1 years of high statistical significance in the years from 1826 to 1837, which accounts for 84% of the overall variability around a time structure or chronome-adjusted mean (of 153 ± 12.6 [SE]). Using this period and its linearly computed characteristics as an input for nonlinear rhythmometry, which procedure allows all parameters, including the period, to vary, validates the result from linear rhythmometry. The period is thus pinpointed as being of 8.18 years, for the span examined. What seems critical, with procedures already available before 1838, the amplitude around the mean of 111 ± 17 can be given with its confidence interval, thereby rejecting the null hypothesis that the mean is

constant, which is in keeping with the rejection of a zero-amplitude (no-cycle) hypothesis, i.e., in keeping with an amplitude different from zero, Figure 3.

Only in 1844 (under the apparently misleading title "Solar observations in 1843") did Schwabe explicitly refer to a cycle (32):

> If one compares the number of days with and without sunspots, one finds that the sunspots undergo a periodicity of about 10 years, and that the spots accumulate during 5 years so frequently that in this time span there are few or no spotless days. ... The future must teach whether this periodicity can be confirmed and whether the minimal activity of the sun in producing the spots lasts one or two years, and whether the activity increases faster than it decreases.

In 1845 (33), Schwabe wrote with even more conviction: "The periodicity *[sic]* of spots of about 10 years, which I had documented earlier, is confirmed by this year's observation". He properly adds that the almost perfect correspondence of the numbers in 1834 and 1844 is entirely due to chance. In 1849 (34), Schwabe used his data for prediction: "If based upon the by-now 23 years of observation, a periodicity of sunspots of 10 years would continue to occur, then the diminution of 5 years would follow after 1849 and thereafter an increase in number until the year 1858" (cf. 35, 36).

Arago (1786-1853)

In 1855, Dominique François Arago's posthumously published *Popular Astronomy* described both kinds of Schwabe's tabulations, the number of spotless days and the numbers of groups of spots (54). Arago was a close friend of Alexander von Humboldt, who had included 25 years of Schwabe's sunspot data (1826-1850) in the third volume of his work *Cosmos*. In the early 1830s, Arago had written Humboldt a letter in which he was extremely excited about finding periodicity along the 24-hour scale in geomagnetics (46, 55, 56). Arago went into quite a bit of detail in his letter to Humboldt explaining why the within-day variation he recorded was not an artifact. Returning to sunspots, our re-analysis, with the fit of a 10-year curve, the period indicated by Schwabe, shows a statistically highly significant periodicity, Figure 4. Without any analyses, Arago, himself a brilliant mathematician, cautiously wrote that "it seems to follow from the observations of Mr. Schwabe that the appearances of sunspot groups are subject to a certain periodicity; that after they have accumulated during 5 or 6 years their number decreases during approximately the same time span". Arago does not consider, as Schwabe did earlier, an asymmetry between the ascending and descending solar cycle stages; but he refers to a cycle of 10-12 years, rather than to one of fixed length. To Schwabe's merit, he considered the asymmetry 11 years before Arago, whereas Arago, while ignoring the possibility of asymmetry, allowed for variability by referring to a "periodicity of" 10 to 12 years.

Arago was rightly impressed by the observation that "after observations by Mr. Schwabe, the number of days during which the sun is devoid of spots is zero during the years near those of maxima, whereas it increases to more than 100 during the years of minima." This corroborating periodicity is seen in the data tabulated by Arago from Schwabe's observations. As expected, the curves best fitting the yearly numbers of sunspot groups and of spotless days show the anticipated very great difference in phase. Asymmetry in the solar cycle may account for the deviation

from a precise antiphase, whether one looks at the original table in the time domain or at our re-analysis in the phase domain, on a polar plot, Figure 4.

How soon to publish: *chacun a son gout?*

Was Schwabe right, perhaps after some hesitation, to place the data during the first 12 years on record, in his Table 1, letting them speak for themselves (30) ? (His hesitation is assumed since he omits any reference to a cycle or periodicity.) Certainly, in time-macroscopic terms he did the correct reporting in 1838 (30), although the tools were available for the re-analyses reported in the foregoing. By 1830, Gauss had published the method of least squares (57). Actually, nearly 30 years before this method was published (in Latin at the urging of French mathematicians who esteemed Gauss highly, having heard about him from Humboldt, and who did not read German), the least-squares approach had been used by December 31, 1801, for the then-sensational and successful tracking by Olbers of Piazzi's lost asteroid Ceres. Olbers found the asteroid exactly where Gauss had predicted it to be by the method we use to analyze unequidistant data (52, 53), which are frequent in biology when a human observer is involved.

Schwabe wrote about the periodicity of sunspots explicitly in 1844, with 18 (not 17; cf. 50, 51) years of observation, as also clarified before us by Alan Julian Izenman, whose much broader reviews of Wolf and Wolfer (35, 36) are highly recommended reading. With nearly but not quite two 10-year spans, he actually did have a bit more than a replication of his original 8-year cycle. Alternatively, should he have waited for another several decades, covering at least 6 cycles by actual data, using a rule of thumb mentioned by Malin (39)? These are rhetorical questions, since he would also have needed some means for prolonging his lifespan. (Were this not the case, Sabine would have had no reason to propose him for corresponding membership in the Royal Society in 1865 [45].)

In any event, Schwabe was unlucky in his happening to examine a particular sequence of twelve consecutive years' data. Figure 5 shows a time plot on top and a moving nonlinear least squares analysis of the Wolf numbers for 12-year intervals from 1749 to 1947 at the bottom. The periods starting between 1823 and 1826 are the shortest cycles. At the other extreme, had Schwabe been able to start observing about 1789, he might have reported a period of 20 years. He was lucky to err on the less deviant end, i.e., he happened to start his observations at the lesser of two kinds of outlying cycles, at the shortest rather than at one of the longest. Figure 6 shows how variable the cycle length of Wolf numbers can be, varying between 8 and 16 years even when the period is estimated by nonlinear least squares based on yearly data in an interval of 35 years, progressively moved by 5 years throughout the data series. Considering a shorter interval of 12 years corresponding to the span available to Schwabe in his first report, Figure 5 shows that the cycle length reached a minimum around 1826 when Schwabe made his observations. If longer data spans are nonlinearly examined, the extremes are reduced but the variability is still substantial, as shown for 35-year intervals in Figure 6.

It is of lexicographic interest that in two prominent encyclopedias, both the publication date of the original communication and the extent of data provided on a topic to make a point continue to be wrong, although the error was noted as far back as 1983 (35). It is also of interest that apparently the two errors are

perpetuated perhaps because neither lexicographers nor scientists could afford what has become a luxury, namely the consultation of original publications, notably since they may have been in a language foreign to the lexicographer.

With time qualification, short series or a single datum can serve

Lexicography aside, several critical points can be made:

First, by now, the history of the sunspot cycle covers five centuries, accounting for observations before as well as after Schwabe's time (58, 59). A very large variability notwithstanding, in a historical perspective, the Schwabe cycle still recurs across centuries with effects in the biosphere (1-9, 22-25, 60-63). The about 10.5-year cycle is not alone; solar variability has many more components than either Schwabe or Arago anticipated. The distinct, now-anticipated about 10.5-yearly or circadecadal cycle is just one spectral component of variability among several other spectral features of variation, further including trends, some of which are cycles with longer and longer periods, constituting parts of the sunspots' chronome.

Second, whenever a short data series or even a single observation is being studied (64), it is useful to time-code first and then to place the interpretation of the datum into a broader temporal context, into a so-called time horizon (65). Thus, with prior mapping of circadian characteristics, a single timepoint can be strategically specified for comparing two groups, such as one of mice at 3 weeks after blinding vs. one three weeks after a sham-operation (64). By knowing that the blinded group's natural desynchronized periodicity is shortened by about 30 minutes, while the schedule of the mice with eyes is 24-hour synchronized by the common environmental lighting regimen, one can anticipate and validate a phase difference predicted for a given desynchronized group, at minimal cost and effort. One needs only the foregoing time horizon, i.e., the extent of shortening of the period, to anticipate the extent of deviation from 24 hours in period length after a given procedure. This demonstration (64), submitted on a single sheet of paper, led to the selection of one of the authors for a Biosatellite study (66). His competitors at the time had submitted bound volumes of proposals, without, however, accounting for time horizons. Many useful, cost-effective predictions about timing can be made in the light of chronomes, while much waste continues without the use of system chronomes and chronome horizons, Figure 1.

Third, there is no general rule that an observation at a single timepoint (64) or data over a single cycle should be ignored (39). This point is doubly important when a biological cycle covers a sizeable portion of a human lifespan.

Fourth, there are cycles that a priori cannot be replicated for the given individual, such as those in natality, morbidity and mortality, that can provide invaluable information about environmental effects upon human growth in health (6, 67) and upon risks of heart and brain disease (6, 68-71), including suicides.

Transdisciplinary challenge

The engineer who usually deals with 50 or 60 Hz and the cardiologist who studies the heartbeat can expect to encounter an about 10-year rhythm, for which the sun may be guilty. In the same way that blackouts may be associated directly or indirectly with magnetic storms, with a periodicity of about 10.5 years, so solar cycle number- and stage-dependent myocardial infarction may also be of interest to the cardiologist focusing upon more than the frequency of 1 Hz. Before

countermeasures can be planned, the transdisciplinary task for meteorologists and cardiologists of the future is, respectively, to provide and interpret a (now routinely Internet-provided) report on space weather and to recommend countermeasures with an efficacy yet to be proved by studies carried out under controlled conditions. Co-designs by epidemiologists may serve to find out what the pertinent variables are, and for physicians to see whether drugs or other measures can prevent undesired effects (6, 72).

In all these tasks, we are dealing with probabilities, hardly ever with certainties. The best rule of thumb is that apparently used by Schwabe and Arago: the systematic sampling over a first unanticipated cycle is better than single observations, even though, as information becomes available, a strategically placed single set of determinations can also provide critical information (64). Obviously, along the same line of thought, sampling over two cycles is better than over one. Thus, after documenting two cycles, Schwabe was almost certainly convinced that it was possible to use the data for prediction, and he did so. (One of the authors was once the dinner guest of a professional statistician, now deceased, who was both a gracious host and a fine professional. The latter asked about minimal sampling requirements in chronobiology [73]. The author replied by joking that two cycles are better than one, and three better than two, thereby wanting to indicate, presumably with Blaise Pascal, that inferential statistics are common sense [read: chronomics] applied. The host, with whom we gladly cooperated [73], took the joke seriously. The "rule" of three cycles being needed in biomedicine thus emerged to compete with a larger minimum of a geophysicist (39). The actual rules are that each single datum, if chronome-specified, gains from its interpretation in the light of a time horizon on the one hand, and that there is no alternative to continuous surveillance for complete information on the other hand.

Apart from astro-helio- and geophysically influenced states of health or disease, in basic science as in emergency medicine, before giving a clean bill of health, we should proceed cautiously as Schwabe did, and check out variability. Cognizance of chronomes with their maps in system time(s) available are to be checked and summarized to constitute time horizons. Time horizons are indispensable for an interpretation of dynamic variables as part of diagnostic thinking, just as much or actually much more so than an exclusive reliance on the fiction of a true representative value. It will have to be explored how best to replace the initial single sample, the imaginary baseline (74), or a single cycle or even 3 or 6 cycles, e.g., in search of a "true", for instance, blood pressure (75) by the sooner or later indispensable continued monitoring. As technology develops, whether we deal with sunspots or health care, or we confront interacting chronomes, strategic if not continuous yet coordinated monitoring is possible systematically, even by self-measurement (76, 77). As we can retrieve information from vehicles in space, we can coordinate this current governmental endeavor with systematic, also governmental physiological and physical and archival data collection in order to gain, from the data collected in calm times, reference values for warnings. By detecting the earliest alterations, we can use them for the timely endeavor of catastrophic disease prevention.

Prejudice against cycles, then as now

Schwabe's example probably shows how two errors, the publication date of 1843 (rather than 1844 or 1838) and reference to 17 (rather than 18) years of observation, copied by one person after another, can be perpetuated. There are different kinds of errors, of course, and the worst are based on prejudice. Gauss, the prince of mathematicians, apparently fell prey to such prejudice. Reportedly without examining Niels Abel's proposition of the insolubility of quintic equations, he dismissed it as "another of those monstrosities". Thereafter, so far, it reportedly proved to be correct. Much thorough and ample chronobiologic evidence in the fields of many opinion leaders continues to be dismissed as too complex, too expensive or both; the wealth of information showing that at least a week of data is needed for screening blood pressure is a case in point (78-80). Current automatic as well as self-monitoring requires patient education. But ignoring variability by focusing on the "correct" single measurement may lead to greater problems than all of those commonly considered (81; cf. 78). The store of information on variability, accumulating as chronome maps in physics as in biology, must be used. The greater error prevailing today in most places, except in a few places like Jarmilka's Brno, was described by Manuel J. Johnson, president in 1857 of the Royal Astronomical Society, as he awarded the Society's medal to Schwabe in absentia (45):

> He [Schwabe] could hardly have anticipated the kind of result at which he has arrived; at the same time we cannot imagine a course of proceeding better calculated for its detection, even if his mind had been prepared for it, than that which he has pursued from the very commencement of his career. Assuredly if he entertained such an idea, it was not borrowed from the authorities of the last century [author's note: including Galileo and Newton], to whom the solar spots were objects of more attention than they have been of late years.
>
> "Nulla constanti temporum lege apparent aut evanescunt," says Keill in 1739. - *Introduct. ad. Physic. Astronom.* p. 253.
>
> "Il est manifest par ce que nous venons de rapporter qu'il n'y a point de regle certaine de leur formation, ni de leur nombre et de leur figure," says Cassini II. in 1740. - *Elém. d'Astron.* vol. i, p. 82.
>
> "Il semble qu'elles ne suivent aucune loi dans leur apparitions," says Le Monnier in 1746. - *Instit. Astron.* p. 83.
>
> "Solar spots observe no regularity in their shape, magnitude, number, or in the time of their appearance or continuance," says Long in 1764. - *Astron. vol.* ii., p. 472.
>
> "Les apparitions des tâches du soleil n'ont rien de regulier," says Lalande in 1771. - *Astron.*, vol. iii, § 3131, 2d edit.
>
> And Delambre's opinion may be inferred from a well-known passage in the third volume of his Astronomy (p. 20), published in 1814, where treating of the solar spots he says, "Il est vrai qu'elles sont plus curieuses que vraiment utiles."
>
> I cite these passages to show, whatever might have been Schwabe's own view of the matter, that *a periodicity of the spots was not a current idea at the time he entered upon the subject* [emphasis ours: thanks to Jarmilka it is a current idea in Brno at the turn of the 21st century], and therefore that he has not merely developed a law for which men's minds were prepared, but that he has been, to all intents and purposes, the discoverer of it.

In a bomb-damaged library in immediate post-World War II Europe, one of the authors found a motto attributed to Galileo: "Measure what is measurable, and render measurable what is not so" (*Omnia metire quaecumque licet et immensa ad mensuram redige).* During the ensuing decades, the same citation purportedly by Galileo was also quoted by noted scientists. When the senior author found that a historian of science had again attributed the words to Galileo, he wrote the historian to ask for an exact reference. The historian at first sent the wrong citation and reference, which was obviously not pertinent, and when this was pointed out to him, pled eye trouble, promising to send the exact citation and reference after he recovered. Decades later, he still has not replied. Eventually, one of the authors, with help from another author, modified the motto: "*Omnia metire quaecumque licet et immensa ad mensuram* ***tempestive*** *redige*": "Measure what is measurable, and render measurable in time, and thus meaningfully, what is not yet so." Since we invariably in science and human affairs rely on the work of others, we add: "Check what is available, and render checkable by re-analyses, what was not yet checked by the original contributor, and do so in time and hence meaningfully."

Herein, we have checked what Schwabe found by a procedure introduced earlier by Gauss, what the splendid mathematician Arago reproduced from Schwabe as such, without using least squares for curve-fitting, although this very procedure had earlier been in the limelight of science. The same Gauss, with Weber, on occasion relied on the "harmony of curves" (82; cf. 83). We recommend the same checking of invisible solar effects, which may bear on problems dealing with growth, suicides and myocardial infarctions as well as with religiosity and criminality (2, 4, 6, 9). Rhythmic changes, once they are mapped, like the sunspots and their associations on earth, maintain a "variable character", even when 500 years of data are available for analysis of economic cycles or 2,500 years in the case of international battles (9). Periodicities longer than about 10 years in sunspots and on earth have emerged. Regarding happenings on the sun itself, in the 20th century, George Ellery Hale (84) reported a double polarity sunspot cycle of about 21 years. We have found its numerical counterparts in different aspects of the biosphere (9).

Concluding remarks

This note is presented with the hope that it can lead to the systematic provision for analyses of data relating to natality, morbidity and mortality, as well as to religiosity and other sociological endpoints, all of them as time series (85). For retrospective analyses, these records should be long enough covering centuries or rather millennia, so as to allow the exploration of 50-year and longer cycles (9), whose documentation is currently in the position of Schwabe's reports in 1838 and 1844, of covering hardly more than a single cycle. The expense of coding these invaluable data for analysis is trivial by comparison to the information they can provide. There is the added handicap that some of the data are very noisy time series. We do not know whether cycles in some of the variables based on only one cycle or others on five or more cycles can be replicated. In the case of Acetabularia, we are in the same position in which Schwabe was in 1838, our samples covering barely more than a single decadal cycle. In other cases, we have to be particularly cautious in reporting on the 50-year component characterizing a very noisy series of international battles that cover the past 2,500 years (9). An about 50-year cycle

characterizes murders in the USA, another ill of society, in the forefront since the terrorist attacks of September 11, 2001.

Whether we deal with the cosmos, with societies or with individuals, there is no substitute for what Schwabe did in dealing with sunspots. In this spirit, rigorous monitoring is ongoing for blood pressure and heart rate in a hospital in Brno, setting an example for the world as a whole. Thank you, Jarmilka, and let us thank your team for giving a new historic aspect to contributions from historical Brno, for leading the way from a medicine and broader science based on spotchecks to one based on time series. Chronomics resolves the dynamics involved in the heretofore neglected everyday range of physiology, economics and all human endeavors. We can measure, in order to focus on revelation, experimentation or both. The difference between the two rests in our minds as distinguished physicists try to document it from the viewpoints of their science (86, 87; cf. 88). Once we analyze the measurements, we also find that "the sun is guilty" (14), not only by what it does now, but also by Mendel's genetics.

Thus, we arrive at questions of ethics. A major question is whether we need ethics committees 1. to enforce long-term monitoring as needed rather than to hinder it, as they managed to do in blessed ignorance in Minnesota; 2. to decide whether universal monitoring should be a public service to safeguard the circulation just as we police the streets, or whether the monitoring should be privatized since people have difficulty accepting the merit of a service offered free of charge. Brno is lucky since it has the country's minister of health to decide the issue.

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Figure 1. Top: two curves differ in period length to a very small extent, as can be the case for circadians in two groups of mice (64). Such desynchronization applies to circaseptan rhythms as well as to circadecadal rhythms, and the abscissa shown in this graph in days could be in months or years for rhythms with longer periods. The figure demonstrates that if one samples at a fixed clock-hour (or in other cases at a fixed day of the week, month of the year or 1-year intervals, as in some aging research), as one compares two groups of which one differs in period, one can obtain monophasic responses *on the same data set* that can be a decrease followed by an increase (at noon) or an increase followed by a decrease at midnight, whereas at other times one obtains biphasic responses (e.g., at 6 a.m. or 6 p.m.), that can be an increase followed by a decrease or a decrease followed by an increase, respectively, again depending on a time fixed usually by convenience rather than pertinence. One can obtain some other nonsensical patterns while believing that one has "controlled" for periodicity as a confounder by fixing the sampling time along one or the other scale. Any research done without knowledge of the time structure involved runs the risk of providing nonsensical results, a basic consideration in any measurements, clinical or experimental.

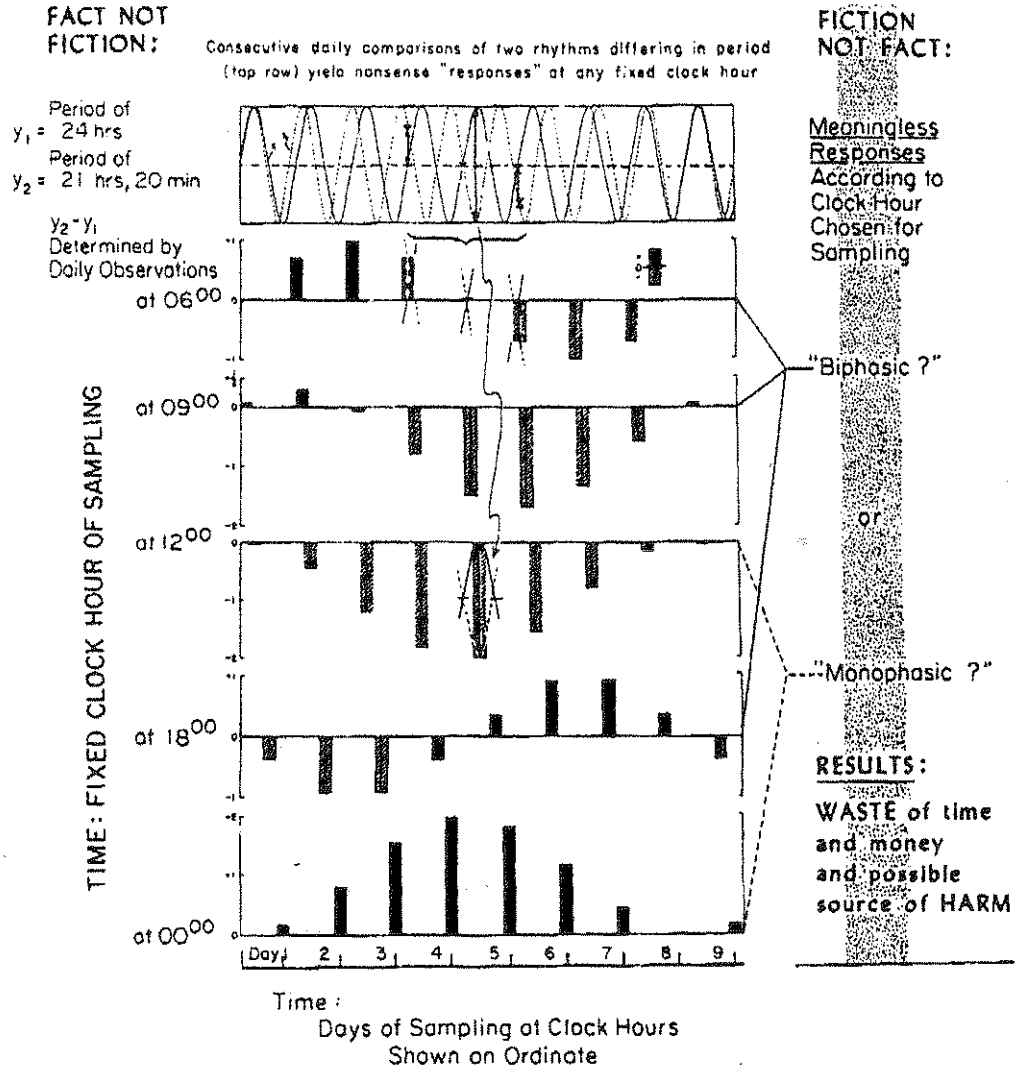


Figure 2. Samuel Heinrich Schwabe (1789-1875). Portrait on website of the High Altitude Observatory, www.hao.ucar.edu/public/education/sp/images/schwabe.html. For further information, the interested reader is also referred to the website of the Red Hill Observatory (Christof Plicht, webmaster), www.plicht.de/chris/32schwab.htm.

Figure 3. First 12 yearly sunspots values recorded by Schwabe (1826-1937), to which a cosine curve has been fitted by nonlinear least squares, using a trial period of 10.5 years.

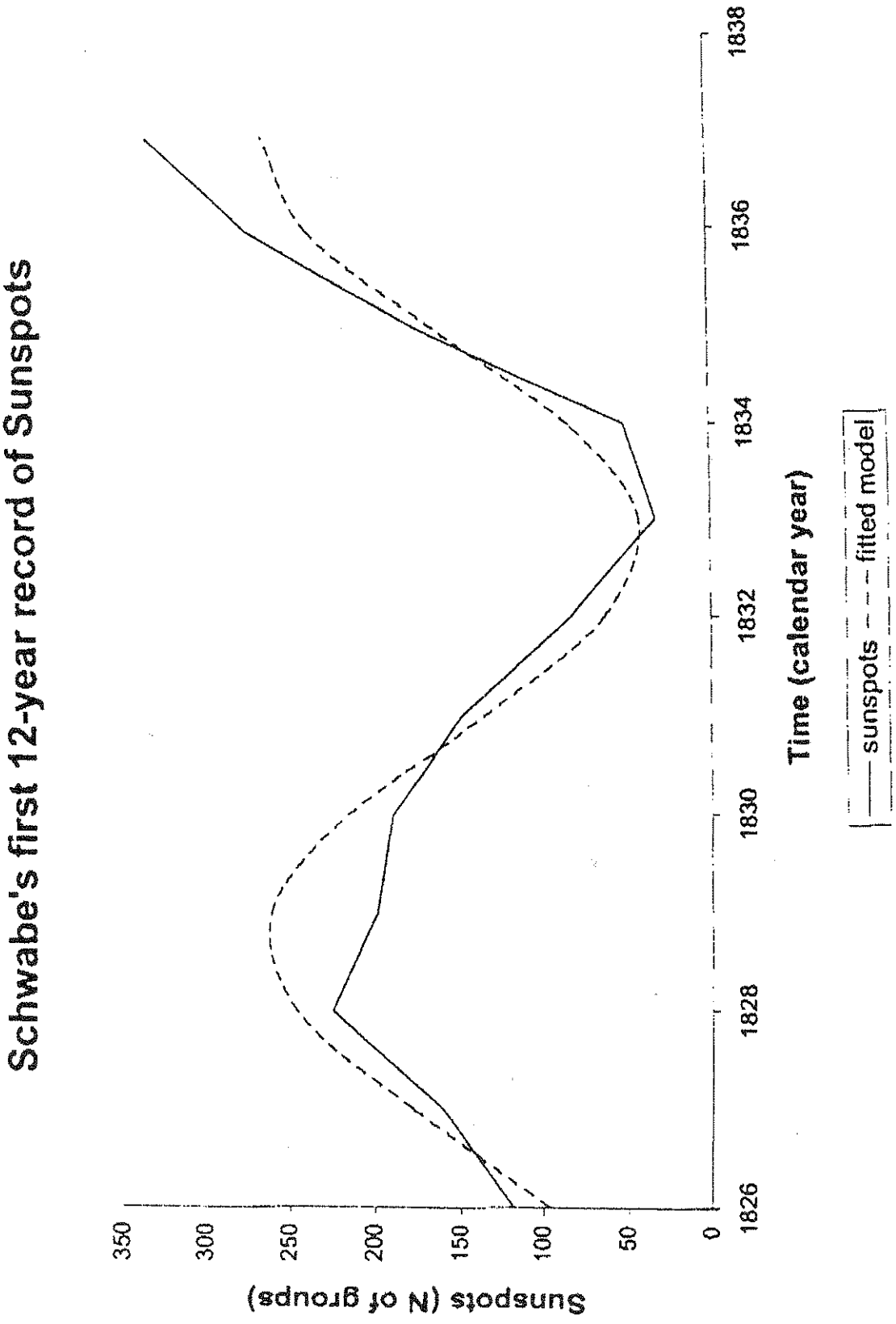
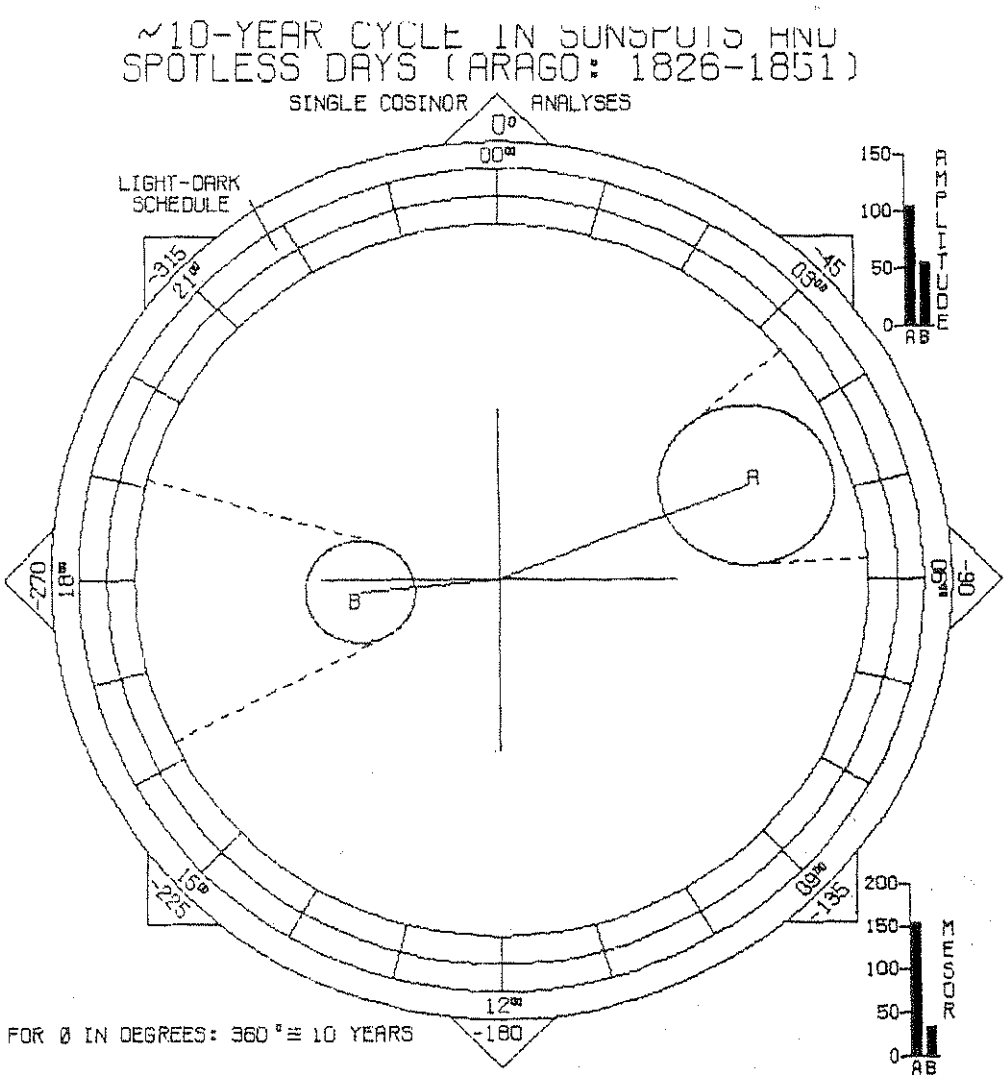


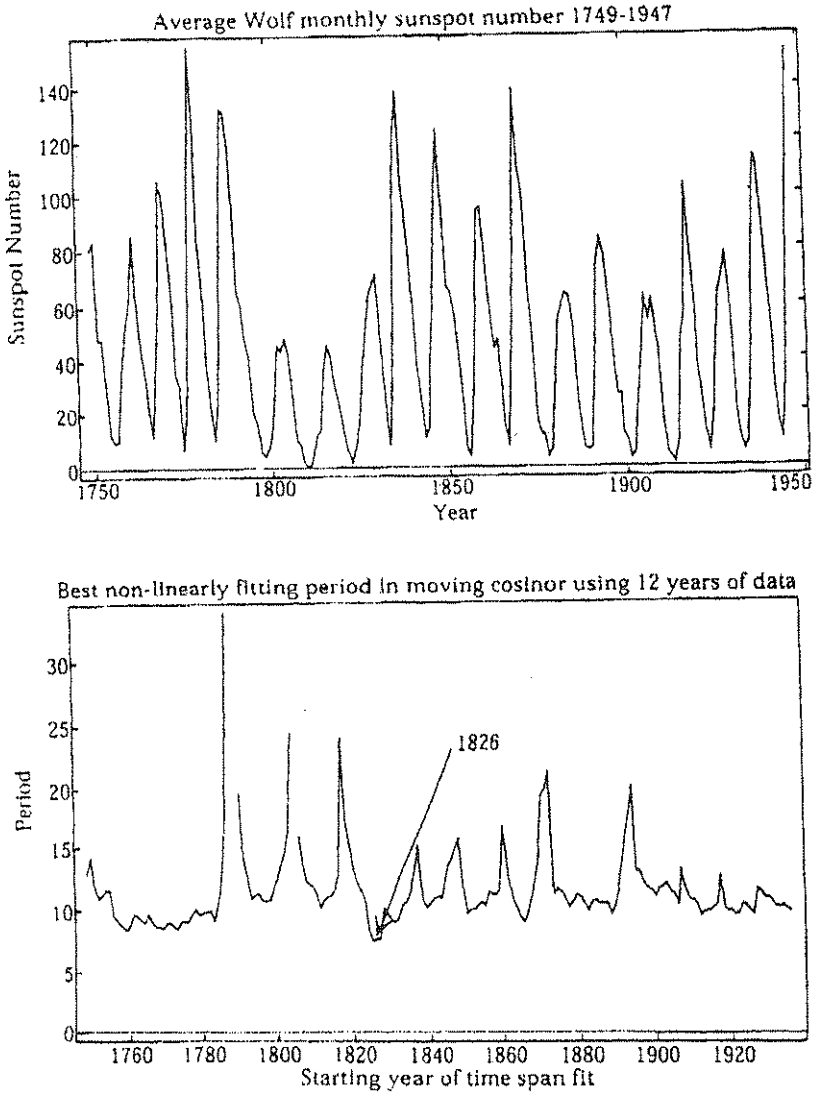
Figure 4. Polar display of cosinor results of trial period of 10 years for the number of sunspots (A) and the number of spotless days (B) recorded by Schwabe and reproduced by Arago, who noticed that the two variables varied nearly in antiphase. After reversing the phase of one of the two variables, parameter test validates the phase difference, yielding a P>0.10 in a test of equality of acrophases. The small numerical deviation from precise antiphase may be accounted for in part by different asymmetrical waveforms.



KEY	P	N	PR	MESOR +-SE	AMPLITUDE *	ACROPHASE (Ø) *
A SUNSPOTS	0.001	26	74	152.72 9.19	105.48 (71.06 139.92)	-69° (-50 -87)
B SPOTLESSDAYS	0.001	26	66	34.53 5.72	55.37 (33.79 76.95)	-265° (-243 -286)

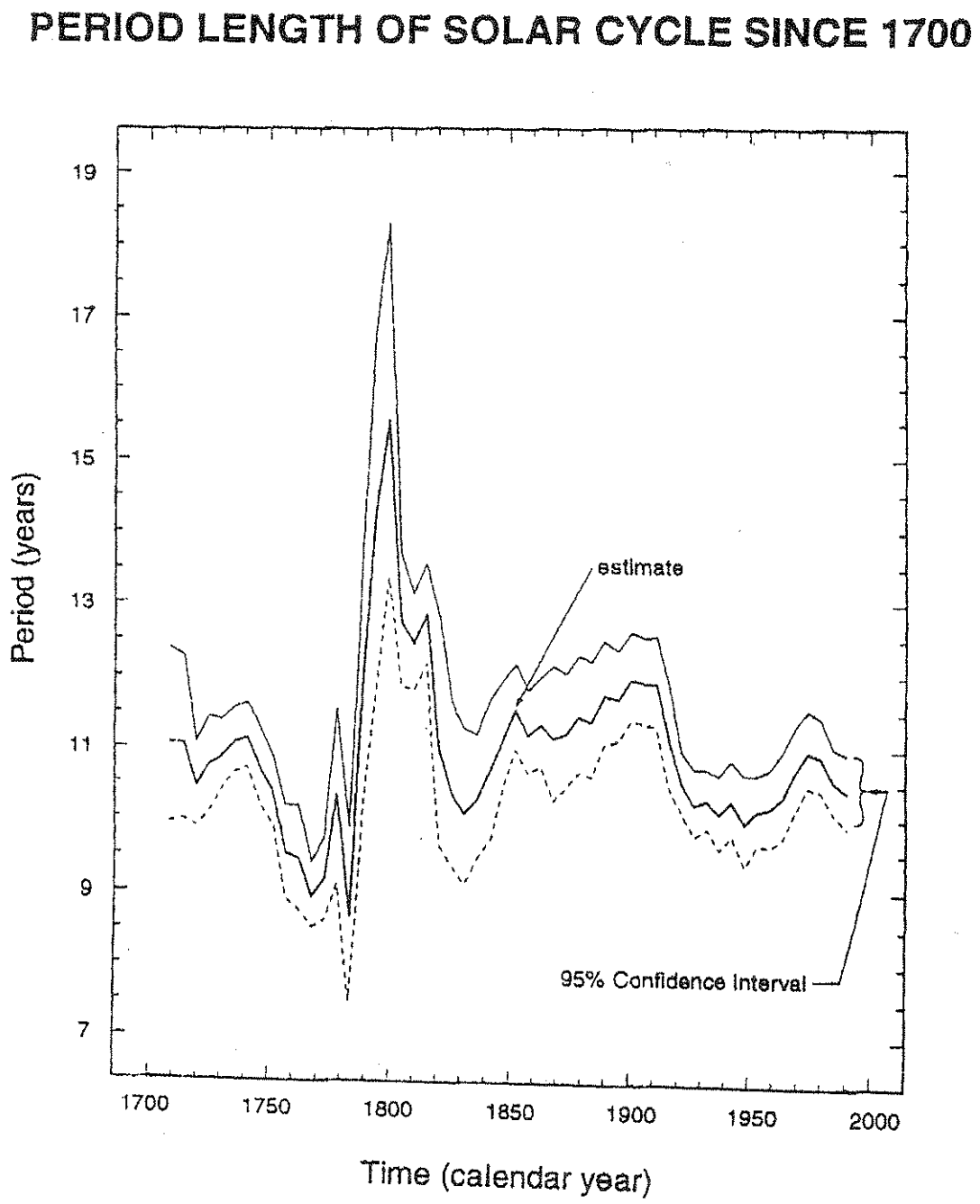
P = PROBABILITY OF HYPOTHESIS: AMPLITUDE=0 ; N = NUMBER OF OBSERVATIONS
PR = PERCENT RHYTHM (PERCENTAGE OF VARIABILITY ACCOUNTED FOR BY COSINE CURVE)
* CONSERVATIVE 95% CONFIDENCE LIMITS (PARENTHESES) DERIVED FROM COSINOR ELLIPSE
CHRONOBIOLOGY LABORATORIES - UNIVERSITY OF MINNESOTA - MINNEAPOLIS, MN 55455 (612)-624-6976

Figure 5. For measurements starting in 1826 when Schwabe made his observation, the cycle length of Wolf numbers extended nonlinearly based on a nearly 12-year interval, corresponding to data available to Schwabe in his report, happened to represent a minimal length in the series thus re-examined by objective means. Had Schwabe examined 12 years of sunspots during the spans starting at times marked by *, instead of finding the shortest period, as he actually did in 1826, he might have been unluckier yet, by extrapolating to very long periods or mistaking the cycle for a trend. His waiting to write about periodicity until he had 18 years of data thus was justified, yet even with 35 years of data shown in Figure 7, the extreme periods can be very greatly deviant. This fact constitutes a lesson for the study of circamultidecadal periods based on short series.



Best period estimated by moving nonlinear least squares cosinor fits using 12 years of data. The starting values were the periods of best fit from linear least squares cosinor analyses at 50 equally spaced frequencies between 1/35 and 1/6 cycles per year (periods 6 to 35 years). The gaps at years 1788, 1789, and 1805 near the start of long cycles indicate the fit did not converge in 100 iterations.

Figure 6. Nonlinear estimate of the cycle length of Wolf numbers analyzed in intervals of 35 years progressively displaced by 5 years throughout the time series.



SCALING, AGEING, BIOLOGICAL TIME AND THE SIGNIFICANCE OF OSCILLATIONS

Thomas Kenner

Institut für Physiologie der Karl-Franzens-Universität Graz

Address correspondence to:

Prof. Dr. Thomas Kenner
Institut für Physiologie der Karl-Franzens-Universität Graz
Harrachgasse 21
A-8010 Graz

Jarmila Siegelova has in many studies contributed important results to the knowledge of the chronobiology of the cardiovascular system. On the occasion of her 60-th birthday I dedicate to her some overlook about problems of time and time-related phenomena in physiology.

SUMMARY

The measurement of time is based on external and internal phenomena. These phenomena have something to do with periods, cycles and oscillations. External phenomena are the daily periods of sunlight and darkness, the weekly periods, the four-week periods related to the appearance of the moon. Also the year-period and certain multiples of this period have to be added to this list - like the 11-year cycles of sunspots.

In biological systems such external cycles have been internalised by the development of biological clocks which synchronise the functions of the system.

In addition biological systems have internal time-related functions which have interesting correlations to the size of the system. Here we will discuss the scaling functions particularly in mammals. One can say: the smaller an animal, the shorter are its internal time periods, including e.g. heart-period and lifetime. Therefore, ageing is a size-dependent process.

Several theories try to explain the process of ageing by genetic determinants, by genetic or metabolic defects, by immunologic failure or by other reasons. It is noteworthy that the duration of life is correlated to the size of the body of different animals. This fact indicates a close relation to metabolism. It can be assumed that under optimal conditions of life, defects in function or structure are repaired by special maintenance functions. During the process of ageing these functions lose effectivity.

It seems that the organism tries to achieve an optimisation of these maintenance functions. Optimisation is defined as a process by which the organism tries to minimise biological cost functionals.

Loss of optimality contributes to instability in the most sensitive periods of life: early development and old age. The concept of optimality and competence includes

the need to stabilise vital functions by external aid. In addition to biological aspects of ageing, social and psychological factors have to be considered.

TIME AND AGE

We all depend on the continuous flow of time. For all macroscopic physical phenomena in biological systems time can be considered as independent variable. In other words: All our activities and actually everything on earth are functions of time. Ageing is a time dependent process, which starts at the moment of conception with the development of an individual. Ageing is a steadily proceeding function of time and ends with death, a catastrophic and irreversible event.

Although all of us experience ageing and time, nothing is known what time really is. It may, thus, be permitted to cite the words by the Austrian poet Hugo von Hoffmansthal from the "Rosenkavalier" (1911):

The ageing grand lady, the Marschallin, speaks to her young boyfriend:
" ... Die Zeit, die ist ein sonderbar Ding. Wenn man so hinlebt, ist sie rein gar nichts. Aber dann auf einmal, da spürt man nichts als sie. Sie ist um uns herum, sie ist auch in uns drinnen. In den Gesichtern rieselt sie, im Spiegel da rieselt sie, in meinen Schläfen fließt sie. Und zwischen mir und dir da fließt sie wieder, lautlos, wie eine Sanduhr .....Manchmal steh´ ich auf mitten in der Nacht und laß die Uhren alle, alle stehn. Allein man muß sich auch vor ihr nicht fürchten. Auch sie ist ein Geschöpf des Vaters, der uns alle erschaffen hat....."

In this poetic description the fact is implicitly included that time can be seen as a physiological, biological and, especially, as a subjective event.

THE ARROW OF TIME

Thanks to the work of Maxwell, Boltzmann and Max Planck, we now know that the irreversible flow of time has something to do with entropy. And entropy is related to the time-dependent increasing probability of the condition of a closed system. The steady increase of entropy means loss of order, increase of confusion, decrease of information and reduction of useful energy. An interpretation of the second law presented by Sir Arthur Eddington can be expressed as "entropy is the arrow of time".

The term Entropy was coined in 1868 by Richard Clausius (cited in Rifkin 1980), and can be described as "a measure of the amount of energy no longer capable of conversion into useful work". The so-called second law of thermodynamics can be summarised by the sentence: the entropy of a closed system can only increase with time.

Decrease of entropy needs external energy and permits an increase of internal organisation, which can be expressed as increase of negentropy (Talbot and Gessner 1973). Negentropy therefore, describes a contribution to information and value (Brillouin 1964). Life is only possible through continuous generation of negentropy.

Ageing can be observed in nonliving and in living systems. Of course, in nonliving systems, the term ageing in a certain sense is used as a metaphor. Living systems include cells, individuals, groups and social systems. Life means that the

increase of entropy is counteracted by systems, which increase negentropy, like biological control systems and by metabolism.
Ageing has something to do with increase of entropy and the loss of proper use of energy.

Power is the rate of use of energy, or work per unit time, and is equivalent to the term metabolic rate in a living system.

Efficiency relates performed work to used energy and thus describes the proper use of energy resources by a system. High efficiency means that in a system the energy provided by fuel or by some energy stores is used economically for purposeful work.

On the other hand, effectiveness is a purely outcome-related value. One can reach the same outcome with high or low efficiency (Ellencweig 1992, Dezsy and Schwanzer 1993,). It seems logic that under normal conditions high effectiveness should be related to high efficiency. It seems, however, that the process of ageing leads to a reduction of efficiency. As long as possible, however, the organism attempts to keep effectiveness as high as possible.

In living systems structure and function are supported by control mechanisms (Milsum 1966, Attinger 1970) and maintenance mechanisms (Holliday 1990). The normal function of a control mechanism is stable. Stability means that the system permits to correct effects of external or internal disturbances and thus to keep the controlled output corresponding to the reference input.

Any uncontrolled deviation of the output from the reference input indicates instability. Many phenomena during ageing can be explained as effects of instability. An example related to age-dependent changes in the arterial system is the continuous stiffening of the arterial wall, which leads to a consecutive increase of the pulse wave velocity and also to the general trend towards an increase of the systolic blood pressure (Wetterer and Kenner 1968, Kenner 1979).

Maintenance functions (Holliday, 1990) are mechanisms which act to maintain the adult in a healthy state for a considerable proportion of the total lifetime. And, of course, each of these functions and mechanisms includes a whole set of control mechanisms:

Regeneration and healing
DNA proof-reading and repair
Removal or repair of defective proteins
Removal of free radicals
Removal of toxic chemicals
Immune response
Control of internal milieu
Stress response (e.g. through heat shock proteins)
Etc.

We can assume that all the control- and maintenance-functions in a normal healthy person have the capability of optimisation. This means that all the variables and parameters involved are adjusted in such a way as to increase the efficiency of the whole system or/and of subsystems.

STABILITY AND OPTIMALITY

In order to try to understand the role of optimality of control- and maintenance mechanisms in ageing it is necessary to consider that ageing is characterised by multiple declines or failures of these mechanisms of functions. These multiple defects then may lead to overt instability.

The time periods after birth and before death represent two critical phases of life where failure and instability may easier be induced than during the main part of life. Yates (1993) explains these phases of instability for a human being with respect to the level of "minimal stability for system autonomy". It may be mentioned that phases of low stability or of instability may also appear at times of major transformations such as puberty.

Medicine is still focussed very much to pathology or defects in order to grant quick interventions. Recently, however, the concept of optimality and the concept of competence are combined with the idea of prevention, guidance and aid.

The concept of optimality has been developed by Heinz Prechtl (1980, see also Perat 1993) for the phase of low stability during early human development. Prechtl uses the term optimality in the sense of a score, i.e. a sum of 42 yes (1)- or no (0)-decisions corresponding to the presence or absence of normal or ideal conditions in past or presence. Thus, the optimality score is a number, which indicates the closeness of the biological status of the system to an ideal overall condition. The score has diagnostic significance: If the number of non-optimal conditions in the optimality list is high this means that the system (i.e. the baby) is in danger because it exists below the limit of "minimal stability for systems autonomy".

In the historic development of concepts for old age the earlier defect-model has given way to a concept of competence (E. Olbrich 1990). This means a preference of looking at the existence of optimal conditions. Competence is defined as the ability to act within the individual limits of undercharge and overcharge (L.Rosenmayr 1989, cited by Olbrich 1990). The list of optimality items has to be defined within the scope of the WHO-definition of health: physical, psychological and social factors.

The process of optimisation minimises a cost functional by adjusting system parameters to values within a range, which depends on momentary individual conditions. Typical examples for optimisation in the circulatory system are the minimisation of material, volume and energy consumption and, apparently, the continuous readjustment of the necessary parameters of the system during sleep.

Whereas the optimality concept is mainly related to the history of the system, optimisation adjusts the current or future conditions to the best possible compromise. This means that even under condition of non-optimality the organism still attempts to optimise its functions. However, in general the existence of optimisation processes in biological systems is the prerequisite for the generation of normal conditions and for a high score of optimality.

In general, the optimal condition means that efficiency as well as effectiveness has high or maximal values. During the process of ageing efficiency may decrease. Therefore it may be necessary to make a compromise: keep the effectiveness at a high level at the cost of decreasing efficiency. In other words: the limitation of

effectiveness leads to a shift of the optimal condition towards lower values of efficiency.

OPTIMALITY AND OSCILLATIONS

There are many arguments suggesting that biological functions are optimised (Pfeiffer and Kenner 1978, Monos and Szücs 1995). It is extremely difficult to prove such assumptions in specific examples. It can be shown that mathematical algorithms to optimise certain functions yield results which agree well with experimental observation. One example which was studied by Pfeiffer and Kenner (1978) is the characteristic triangular contour of the time-course of the left-ventricular ejection.

It is a matter of fact that most biological functions oscillate. Typical examples are the heart beat, the respiration, the blood pressure. Besides the interesting fact, that several of these oscillations tend to synchronise - which again suggests some kind of optimisation - the question is not definitely answered whether these oscillations have specific functions in the control of biological systems. Monos and Szücs (1995) and other authors suggested that oscillations are necessary to guide a control system according to an optimising search strategy to find the best possible value or path of a variable. These authors presented the blood pressure control system as an example.

If the presence of optimal control strategies in each animal is assumed then it seems plausible, that these strategies have been developed in animals of different size, like mouse or elephant - as we actually observe. As a consequence we find general rules of biological similarity.

BIOLOGICAL SIMILARITY

Biological similarity may be interpreted as the apparent fact that evolutionary selection retains the result of optimisation. E.g.: the assumption of optimisation in the relation between the function of the heart and the properties of the arterial system permits to predict the typical relation between body weight and heart rate.

There are still many other viewpoints concerning the phenomenon of time and time-dependent processes. E.g. we may consider the many periodic events which can be found in physiological functions. We are continuously involved in daily, monthly and longer periods of biological functions. One particular phenomenon has to be mentioned in some more detail: Time periods and intervals, including the maximal lifetime is, in mammals, closely related to body mass and metabolism.

Allometric functions relating ageing, intervals and energy of mammals to the body mass M (kg) according to Schmidt-Nielsen (1984) are:

Pulse Interval (min) = (1/241) $M^{0.25}$

Lifetime (y) = 11.8 $M^{0.2}$

Metabolic Rate = 70 $M^{0.75}$

Specific Metabolic Rate (Metabolic rate per unit body mass) = 70 $M^{-0.25}$

As far as the exact values of the exponents of the allometric functions are concerned there is a discrepancy between diverse statistical estimates and also between statistical estimates and theoretical assumptions. Recently an interesting discussion was stimulated by the attempt to answer the question why the metabolic rate grows with the exponent 3/4 and not with the exponent 2/3. The latter would be expected by the assumption that the loss of metabolic heat is proportional to the surface of the body. West at al. (1997) had suggested that the fractal structure of the vascular distribution plays a major role. Using such an assumption these authors tried to prove the exponent 3/4. In contrast, Dodds et al (2001) reanalysed statistical data sets from the literature and came to the conclusion, "that present theories for exponent 3/4 require assumptions that render them unconvincing for rejecting the hypothesis that exponent 2/3".

Nevertheless, the main conclusions agree between both assumptions. The so-called specific metabolic rate is the larger, the smaller the mass of the animal. The specific metabolic rate of a 30-g mouse is tenfold higher as compared to a 300-kg cow.

The lifetime - and also intervals of rhythmic biological functions like heart beat or ventilation - are shorter the smaller the mass of the animal.

It can easily be calculated from the so-called allometric equations as shown above, that a mouse in her lifetime has as many heartbeats as a cow in a tenfold longer lifetime.

THE HUMAN VALUE

In human life not only the metabolic efficiency is counting, but also psychological aspects and human values. Robert Rushmer (1975) discussed the economic values. The two periods in which a human being, from the viewpoint of economy, is "a drain on family or society" correspond - according to Yates (1993) - to periods of threatening instability. The highest mortality rate can be observed during the first year of life. The second unstable period is the time of old age, and is again "a drain on family or society". To become old - due to the help of modern medicine - is becoming a more and more an increasingly difficult problem.

In order to illustrate the problem of decreasing power and efficiency and the steady increase of entropy - in the sense of disorder and disarrangement - in old age, I shortly comment a concept by Moreno (cited by Petzold and Bubolz, 1979). He symbolises the connectivity of a person by circles. The person has to be seen as the centre; the circles represent the distances of social contact. With increasing age the contacts either get lost by death of friends and relatives or by difficulties to walk or even by loss of interest.

With increasing age the general efficiency of the body in the sense of metabolic and physical as well as psychologic and motivational efficiency decreases. Therefore, with increasing age it becomes less important, if a certain action is performed with a high or low efficiency. The main goal is primarily the outcome. The term effectiveness (in contrast to efficiency) describes any measures, which improve the outcome by - let us say - external or internal aid or help and relates achievement to desire. In other words, the ageing person needs the support of the family and the society.

Olbrich (1990) uses the term competence of age, which corresponds to the behavioural effectiveness in the area of age-specific demands. Instead of focussing particularly to defects of age it seems to be important to optimise age-specific functions. In order to increase effectiveness there are the following possibilities: 1) Give external aid or measures of help. There is a tremendous lack of the most simple daily needs for old people e.g. in the kitchen, in the bathroom, or the ever increasing difficulty to find a grocers store close enough to the living quarter. 2) Adjust the demands to the capabilities. This second internal aid is a question of personality and also of caretaking. This concept, extended to the final stages of life has led to the idea of Hospiz-movement.

In contrast to earlier aspects of old age which were concentrating on negative aspects like defect or disuse, the model of competence, actually follows the concept of optimality as a guide to increase effectiveness by adjusting properly scaled help. Furthermore, it must be concluded that not only physical and metabolical and economic needs of human life have to be provided, but also social, psychological and religious support has to be given according to the requirements of the individual.

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THERMAL REGULATION IN MAN: ITS LIMITS DURING EXPOSURE TO HIGH AMBIENT TEMPERATURE

Martineaud Jean-Paul and Cisse Falu*

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

Address correspondence to:

Prof.Dr.Jean-Paul Martineaud
Service de Explorations Fonctionnells
Multidisciplinaires
Hopital Lariboisiere
2, rue Ambroise-Paré
F-75010 Paris

INTRODUCTION

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

MATERIAL AND METHODS

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

during fasting. We carried out our experiments during winter (moderate climate) and summer (hot season).

The first and second series of measurements on circadian rhythm of central temperature were carried out in Kedougou (western Senegal) during the months of February (moderate ambient temperature) and June (hot period) in normal dietetic conditions. 50 healthy young men (19-26 years old) participated in two trials, for comparison of responses of their circadian cycle in both situations in order to determine the exact influence of two external stresses.

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

RESULTS

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

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

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

body temperatures. The constant value for central temperature independent of environmental temperatures was 37.55°C.

DISCUSSION

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

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

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

this level there was a more efficient reaction of the thermoregulatory system in order to maintain the body temperature in good conditions for tissue metabolism.

CONCLUSIONS

The studies carried out in the intertropical zone during temperate (winter) and hot (summer) seasons demonstrated that the circadian cycle of body temperature was present in all circumstances, but with increase in his amplitude during the hottest season, by increase in the diurnal values, revealing a physiological adaptation of the thermoregulatory system to the environment. This increase was at least partially due to the food intake, particularly proteins inducing a specific thermogenesis. Furthermore a relationship was established between central and ambient temperatures with an upper limit. This limit was approximately 37.5°C for the central temperature, corresponding to 35-37°C for environmental temperatures. For higher values human organism becomes again a strict homeotherm, showing the limits of physiological adaptations to the thermic stress.

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CLINICAL RESEARCH OF THE CZECH MYELOMA GROUP (CMG): THERAPY OF MULTIPLE MYELOMA (MM) WITH HIGH DOSE MELPHALAN FOLLOWED MAINTENANCE THERAPY (MT) WITH INTERFERON ALPHA {IFN} OR SEQUENTIAL MT IFN/DEXAMETHASONE (DEX). (INTERIM ANALYSIS OF THE RANDOMIZED TRIAL "4W" OF CZECH MYELOMA GROUP).

Vorlíček Jiří, Hájek Roman, Krejčí Marta, Ščudla Vlastimil, Bačovský Jaroslav, Koza Vladimír, Schutzová Marie, Faber Edgar, Papajík Tomáš, Fraňková Hana, Lehanka František, Stavařová Yveta, Vránová Milena, Kuča Bedřich, Kessler Petr, Cahová Stanislava, Gumulec Jaromír, Pozděnová Věra, Walterová Lenka, Štefánek Ivan, Mayer Jiří, Adam Zdeněk

Address correspondence to:

Prof.MUDr.Jiří Vorlíček,CSc.
Dean of Faculty of Medicine MU
Head
Department of Internal Hematooncology
Faculty of Medicine, Masaryk University
Jihlavská 20
639 00 Brno

Jiří Vorlíček et al. on behalf of CMG

The Czech Myeloma Group was born in 1996 focused on a single purpose. To accelerate the clinical and preclinical research for a cure for multiple myeloma. To achieve this purpose, four goals are pursued: Funding research in the field of multiple myeloma; Building collaboration among researchers; Providing information to patients and family members; Raising awareness of multiple myeloma. The first activity of CMG was the clinical protocol "4W" .

SUMMARY

212 patients were enrolled in the trial 4W from April 1996 to August 2001. Previously untreated MM patients underwent four courses of VAD chemotherapy; priming with cyclophophamide 5 g/m2 and high-dose chemotherapy with melphalan 200mg/m2. 161 pts. underwent autologous transplantation and 151 pts. were randomized into the two arms of maintenance therapy (MT): IFN or sequential MT (IFN for 3 months followed, after 4 weeks pause, by 40 mg DEX in days 1-4,10-13,20-23 and after 4 weeks IFN starts again for the next 3 months).

Overall responses were 71 % after VAD and 79 % after PBSCT with only 15 % of CR confirmed with immunofixation after transplantation. Early transplant related mortality was 2,48 % (4/161) and almost 26 % of patients did not achieved

randomization procedures. 74 pts. and 77 pts. Were randomised into IFN group, IFN/DEX group respectively. Total of 56 relapses (IFN-24/IFN+DEX-32) and total of 25 death (8/17) already occurred in these randomized patients with median of event free survival of 36,4 months for all group with no significant differences between groups (p=0,22) but with evident trend for better survival in IFN group (p=0,09) although median of overall survival was not yet achieved. Results of interim analysis of the trial 4W will be presented.

INTRODUCTION

Multiple myeloma (MM) is a B-cell malignancy that cannot be cured with currently available therapy (1). Oral prednisone and melphalan is still widely used treatment and a meta-analysis of 18 published trials has shown no survival advantage of combination chemotherapy (2). After pioneering work using escalating dose of melphalan without stem cells or bone marrow cells support (3), high doses of chemotherapy followed by autologous transplantation became widespreadly indicated as upfront therapy for newly diagnosed patients with multiple myeloma in the last decade (4, 5, 6, 7, 8). Prolonged event-free survival (EFS) and overall survival (OS) was improved in one randomized trial (9), and also in many non-randomized trials (4, 5, 10) with an estimated benefit of 1-2 years on OS against conventional treatment. Although tandem transplantation is frequently used in many transplantation centers (4), the data from the only one randomized trial became recently mature enough to prove benefit of tandem transplantation (11).

Despite the strong interest of physicians in the evaluation of the curative potential of IFN in patients with MM which have been studied for almost 20 years after conventional treatment, the benefit of interferon-? (IFN) is not clearly demonstrated (12, 13). The meta-analyses performed have demonstrated a mild advantage for IFN maintenance therapy (approximately 6 months after 5 years) on both EFS and OS (14, 15). The first encouraging results of randomized trials using IFN maintenance therapy after autologous transplantation at a median follow-up of 52 months have ceased to be significant after 5.5 years of follow-up (16). Results of the retrospective registry study from the European Group for Blood and Marrow Transplantation (EBMT) support IFN maintenance therapy after autologous transplantation in patients with MM although better results in the IFN group of patients could be involved partly with better prognostic factors in the IFN group (17). A more effective therapy is necessary to affect minimal residual disease and improve the prognosis of patients suffering from MM. New methods of immunotherapy, gene therapy, vaccination with Id protein or dendritic cells and consolidation chemotherapy are being tested in the phase I/II clinical trials (18, 19, 20, 21). An allogeneic approach using donor leukocyte infusions for induction of graft versus myeloma effect is the only one that is able to induce clinical measurable response for limited number of patients (22). Its application in MM patients is limited to less than 10 % of patients due to the advanced age of patients and absence of suitable donors and associated toxicity and mortality (23, 24).

Our routine treatment strategy in untreated MM patients includes autologous transplantation in all suitable patients. The clinical trial "4W" of Czech Myeloma Group (CMG) was started in April 1996 to compare different types of maintenance treatment after autologous transplantation. Twenty-three centers including three

transplantation centers are involved in this trial. The results of trial 4W have confirmed the feasibility of autologous transplantation procedure (25). Monitoring of EFS and OS is in progress. Data from the annual analysis are reported here.

MATERIALS AND METHODS

Patients

Between April 1996 and August 2000, 167 previously untreated patients with stage I-III multiple myeloma according to Durie and Salmon (26) were enrolled. Patients were included according to the following criteria: age less than 65 years (unless special circumstances); stage II-III or stage I with 2 of 3 risk factors of early progression according to Facon et al. (Hb < 120 g/L, plasma cells > 30 %, IgA > 20g/L or IgG > 30g/L) (27); untreated patients (95 %) or pretreated patients with maximum 4 cycle of chemotherapy (5%) without any pause before enrollment; monitorable disease (M-component) in serum or urine; signed informed consent of a protocol approved by local ethical committees. All patients received induction chemotherapy (see below) followed by graft collection (139 patients) and autologous transplantation (130 patients). Patients with cardiac dysfunction (NYHA>II, ejection fraction < 50%) and/or respiratory dysfunction and/or low performance status (Karnofsky < 70%) and/or severe life treating complications during induction treatment or poor graft collection were excluded from transplantation. Until the date of analysis (August 31, 2000), 113 patients have been randomized to maintenance therapy with IFN or IFN and dexamethasone (DEX) as soon as clinical response after transplantation was evaluated, usually 4 weeks after transplantation. Only patients who did not achieve a response better than 75% reduction of the monoclonal immunoglobulin (M-component) during the first months after transplantation were indicated for a second transplantation (10/113), after which they continued in maintenance therapy as randomized.

TREATMENT

Induction treatment

All patients received courses of VAD chemotherapy consisting of vincristine 0.4 mg/day intravenously (i.v.) by continuous infusion for 4 days, doxorubicin 9 mg/m2/day i.v. by continuous infusion for 4 days, and dexamethasone 40mg per os (p.o.) on days 1-4, 10-13, and 20-23. Four courses of the VAD regimen were administered with the interval of four weeks. If the chemotherapy administration was complicated by bone marrow failure, neuropathy, diabetes mellitus, or any other side effect, doses of chemotherapeutic agents or DEX were reduced or stopped as required. Finally, rapid infusion (2 hours) of doxorubicin and vincristine into a peripheral vein was allowed in the patients with difficulties with central venous access.

Peripheral blood stem cell mobilization

All patients received cyclophosphamide 5g/m^2 i.v. divided into 2-3 doses during 36 hours and recombinant human granulocyte colony-stimulating factor (rhG-CSF) (Hoffman La Roche, Switzerland) 5-10 µg/kg with higher dose reserved for irradiated patients due to MM. All harvesting used the Cobe Spectra separator

(Cobe, Denver, USA). Stem cells were at -180o C in liquid nitrogen. When required, it was rapidly thawed at 37o C and reinfused immediately without further processing.

High-dose treatment

Approximately 4 weeks after graft collection, high dose treatment was given consisting of melphalan 200mg/m^2 for first transplantation and melphalan 200mg/m^2 with a high dose of methylprednisolone (1,5 g i.v. for 5 consecutive days), day (-2) - (+2) respectively for the second transplantation if indicated. PBSC graft was given 24 hours after myeloablative treatment. GM-CSF (Leucomax, Schering-Plough, USA) 300-400µg/day or G-CSF (Neupogen, Hoffman La Roche, Switzerland) 5µg/kg/day starting usually day 5-7 after transplantation until leukocyte (WBC) recovery above 5 x 10^{12}/L.

Maintenance therapy

After transplantation patients were randomized to two arms of maintenance therapy. Patients were stratified according to the following criteria: age (3 subgroups), stage (3 subgroups), response after 4xVAD regimen (2 subgroups; cut off level- 75% M. component percentile). Eighteen subgroups were blocked per 4 patients (2 pts. IFN/ 2pts. IFN+DEX). Patients in arm A received IFN 3 x 10^6 units three times weekly s.c. (Intron A or Intron A Redipen, Schering-Plough, USA). Patients in arm B started with the same dose of IFN for 3 months followed by a 4-week pause, DEX 40mg p.o. on days 1-4, 10-13, 20-23, with a 4-week pause. Then the alternating cycle of IFN/DEX maintenance therapy was restarted again. The Schedule of the protocol is in Figure 1.

Supportive Treatment

The bisphosphonate clodronate was recommended only for patients enrolled to the trial. Irradiation of osteolytic lesions (20 %) or site of spinal cord compression (2 %) was done if required, as well as orthopedic treatment or support. Neutropenic sepsis was treated with standard intravenous broad-spectrum antibiotics according to the local hospital guidelines. Supportive measures during transplantation usually included prophylaxis with ciprofloxacin, and fluconazole or itraconazole. Packed red cells and platelets were transfused if required according to local hospital guidelines.

Assessment of Toxicity

Toxicity was assessed according to SWOG criteria.

Criteria for Response

The definition of EBMT/ABMT/IBMT therapy criteria for disease response was used with several modifications (28). Patients were regarded as achieved complete remission (CR) when the original serum M-component had disappeared, tested by immunoelectrophoresis including immunofixation (immunofixation was done at regular interval, at least one per year); no urine M-component measurable; less than 5% plasma cells in the bone marrow aspirate; no new osteolytic lesions or their progression. Patients were in remission (R) if there was a 75 % decrease in measurable M-component in serum and in partial remission (PR) if there was 50%

decrease in measurable M-component in serum or 90% decrease of light chain in urine; no new osteolytic lesions or their progression. Minimal response (MR) was achieved in patients who achieved reduction of M-component in serum between 25-49 % or 50-89 % reduction of M-component in urine. Stable disease (SD) was defined as oscillation of M-component between +- 25 % from starting level. All criteria for CR, R, PR, MR and SD had to be fulfilled for at least 3 months.

Relapse was confirmed from CR only, if level of M-component had increased above 5g/L in serum or above 0.2g/L in urine. Fluctuation of positivity and negativity of immunofixation without increased level of M-component was not considered as relapse and patients were evaluated as CR or R according to the last results. Progression was confirmed from R or less response, if level of M-component had increased above 25 % from starting level, but at least above 5g/L in serum or above 0.2g/L in urine. Results have been confirmed at least by two consecutive evaluations during 6 weeks. New osteolytic lesions or their progression, new detection of hypercalcemia above 2.8 mmol/L were also considered as a sign of progression or relapse.

Statistical Analysis

The equal distribution of clinical stages as a stratifying criterion in treatment arms was compared using the chi-square test. Fisher's exact test was used to compare therapeutic responses after VAD induction therapy. For continuous variables, the non-parametric Mann-Whitney U test was used to compare the treatment arms (29). Survival curves were plotted from the start of therapy to the date of death or most recent follow-up. Event free survival was calculated from the date of first transplantation to the date of relapse, death or most recent follow-up. The Kaplan and Meier method was used, and statistical significance was calculated by the log-rank test. Statistical analyses were performed using the software package *STATISTICA* for Windows (StatSoft Inc. Tulsa, 2000).

RESULTS

Patients Characteristics

One hundred and sixty-seven patients have been enrolled in this trial until interim analysis was done in August 2000. One hundred thirty patients underwent autologous transplantation and 113 patients were randomized to receive IFN or IFN/DEX maintenance therapy. Patients' characteristics are listed in Table 1. Median age was 53.0 years (range 32-65) with 37 women and 76 men. According to the Durie-Salmon staging system, 10% of the patients had stage I, 26% stage II and 64% stage III. According to the type of paraprotein, 22% of the patients had type IgA, 63% type IgG, 1,8% type IgD and 13% Bence-Jones type myeloma. From 167 patients enrolled, 18.1% (25/138) patients dropped out from protocol before randomization of which 9.4 % (13/138) were excluded already during VAD chemotherapy, 5.8% (8/138) after cyclophosphamide chemotherapy and 2.9% (4/138) during the transplantation procedure. Main reasons were as follows: sudden death, pulmonary embolisation, severe infection or progression of disease. The randomization procedure resulted in a total of 113 patients of whom 54 patients were randomized to IFN arm and 59 patients to IFN/DEX arm. The efficacy of stratification procedure was analyzed and results are shown in Table 1.

No significant differences in age, stage and response to VAD chemotherapy were found between the two randomized arms.

Response to Therapy

Response rate after VAD chemotherapy and 1 month after transplantation in 113 randomized patients is shown in Table 2. The response to initial induction treatment (4xVAD) was 8 CR (7%), 54 R (48%), 32 PR (28%). The response evaluated one month after autologous transplantation was 13 CR (12%), 63 R (56%), 24 PR (21%). Twenty-seven patients out of 63 achieving R after transplantation had non-detectable level of M-component with positive immunofixation evaluated in 18 patients only. The second transplantation was indicated in 10 patients (11%) who did not achieve 75 % reduction of M-component during 6 months after the first transplantation according to the protocol schedule described above. Transplant-related mortality (TRM) (day +100) was 3.1 % (4 out of 130 transplanted patients at the date of analysis). That included one patient who died on day +123 due to central neuropathy with the first signs of somnolence starting on day +5 clearly related to the transplantation procedure and the toxicity of melphalan.

Response Duration and Survival, Relapse Rate

One hundred and thirteen randomized patients received maintenance therapy, 54 patients were randomized to IFN arm and 59 patients to IFN/DEX arm. Distributions of age (52.4 vs. 52.5), stage (68.5 vs. 61.0 for stage III), Ig type (18.5% vs. 25.4 % for IgA), ß2-M level (3.60 vs.3.40) and other parameters listed in Table 1 and Table 3 were similar in both randomized groups but the level of C-reactive protein was higher in IFN/DEX group (3.85 vs. 6.50; p=0.026). Total of 36 relapses have occurred, in 13 of 54 patients in IFN group and in 23 of 59 patients of IFN/DEX group. Median of event free survival (EFS; from the date of transplantation to relapse/progression) for all 113 patient was 34.9 months, median of overall survival (OS; from the start of therapy to the death) has not been achieved yet (Figure 2). Only the median of EFS in group IFN/DEX was achieved (28 months) but the EFS (Figure 3) and OS (Figure 4) curves show no significant differences between the IFN and IFN/DEX groups (p=0.078 for EFS; p=0.569 for OS).

Toxicity

The toxicity of induction treatment VAD was evaluated and published previously (25) and it is not described extensively in this analysis. The fact should be pointed out that 9.4 % (13/138) of enrolled patients were excluded from protocol during VAD treatment, which accounts for dominating 52% of all excluded patients until randomization. Also, the number of deaths was higher during VAD treatment (9 patients) than during mobilization (1 patient) or transplantation (4 patients). Thus, this period of the protocol should be considered as the most difficult part despite the fact that the majority of common toxicities concerning VAD regimen did not usually exceed grade II of SWOG criteria.

The toxicity of the cyclophosphamide mobilization in randomized patients using SWOG criteria is shown in Table 4. Five patients (5.8%) were removed from protocol after mobilization, of whom one patient died due to rapidly progressing

Pseudomonas sepsis, in one patient the graft was not successfully collected, one patient had a CNS bleeding due to the low platelet count with symptomatic transient paraplegia, and two other patients with resistant disease had sepsis during mobilization and their performance status was lower than the 70% (Karnofsky) required for transplantation.

The toxicity of melphalan 200mg/m2 used as a myeloablative regimen in randomized patients using SWOG criteria are shown in Table 5. Leukocyte count > 1.0 x 10^9/L was reached after 13.0 days (range 8 - 18) and a median of leukocytopenia < 1.0 x 10^9/L was 6.0 days only. Platelets > 50 x 10^9/L were reached after 14.0 days (range 2 - 28) and median of the thrombocytopenia < 50 x 10^9/L and < 30 x 10^9/L was 5.0 and 2,6 days respectively. A median of 1.6 (0-12) platelet transfusion and 1.6 red (range 0- 7) blood cell transfusion was given. The number of days with fever (identified as temperature higher than 38°C) ranged from 0 to 28 days (median 2.6 days). The most frequent and severe toxicity from all evaluated was mucositis. Thirteen patients (29%) underwent transplantation without mucositis or with a minimal toxicity (grade 0-1 SWOG), 53 patients (47%) had mucositis of grade 2-3 SWOG, and 27 patients (24%) required complete parenteral nutrition and opioid analgetic therapy due to grade 4 of mucositis.

IFN was started usually one month after transplantation at a low dose of 3x10^6 units three times weekly. DEX was used at a dose of 40 mg on days 1-4, 10-13, 20-23 two times per year in the IFN/DEX arm. The majority of patients tolerated IFN maintenance therapy well, with very good compliance and acceptable toxicity (grade 0-1 SWOG in 76 % of patients) without dose modification. The toxicity of IFN consisted mainly of flu-like symptoms, sleep disturbances, and malaise that were usually self-limiting. Twenty percent of patients required dose modification and/or temporary interruption of IFN maintenance therapy mainly due to hematological toxicity (thrombocytopenia). Common side effects with maintenance dexamethasone consisted mainly of several days of sleep disturbances, anxiety or irritability, myalgia, fatigue, mouth sores and indigestion and usually did not require dose modification (grade 0-1 SWOG in 67 % of patients). Seven (6 %) from total of 113 patients had to discontinue maintenance treatment prematurely because of adverse effect such as peripheral neuropathy (1 pt.- IFN), severe hypothyreosis (2 pts. - IFN), depression (3 pts.; 2-IFN/1 pt.-DEX) and hallucination (1 pt. - DEX).

Autologous Graft Evaluation

From 139 patients who underwent peripheral blood stem cells collection all (98,6 %) but two patients achieved successful graft collection at least for one transplantation. One patient died during the procedure and the second patient collected insufficient graft for transplantation. A median of 15 x 10^6/kg CD34+ cells (range 0.9 - 65.7 x 10^6) and a median of 25x10^4/kg (range 8.0 - 403.1 x 10^4) CFU-GM were collected. A sufficient number for at least two transplantations was stored in more than 90 % of patients. In the first transplantation, a median of 3.8 x 10^6/kg CD34+ cells (range 0.9 - 23.2 x 10^6) and a median of 8.6 x 10^4/kg CFU-GM (range 2.8 - 115.3 x 10^4) were used. Only one patient did not achieve sufficient engraftment. She did not recover a platelets count of > 100x10^9/L 6 months after transplantation although her platelets count ranged between 50 and 90 x 10^9/L not requiring transfusion.

DISCUSSION

The addition of autologous stem cell transplantation to the standard chemotherapy in newly diagnosed untreated patients with MM has been considered as a new gold standard (1, 4, 5, 9). Induction treatment is usually started with multidrug chemotherapy which should not contain alkylating agents to preserve stem cells and make a successful graft collection possible (1). In patients with untreated multiple myeloma, peripheral blood stem cells could easily be collected after high-dose cyclophosphamide and G-CSF during one or two leukapheresis procedures. Many other mobilization regimens could also be used to collect autologous graft for at least one or two transplantations (30, 31). Based on our experience, the dose of G-CSF should be economically adjusted between 5µg/kg to 12 µg/kg according to the type of pretreatment and the field of radiotherapy received by individual patients as reported in other studies (32).

Despite widespread use of autologous transplantation in untreated patients with MM the only available data are from the first randomized trial comparing standard chemotherapy versus myeloablative therapy supported with autologous transplantation (9). In the follow-up analysis of the next trial of the INTER GROUPE FRANCOPHONE DU MYELOMA (IFM) comparing single versus double transplantation, double transplantation supported with PBSC was found to improve response rate, EFS and OS. No benefit of double transplantation was found if myeloablative chemotherapy was supported with bone marrow graft (11). Longer follow-up is probably still required to give a definitive conclusion. Similar data from an Italian trial in which 192 patients were randomized (single vs. tandem) are still premature to prove any significant differences (33). In 1996, when trial 4W was initiated, little was known about the advantage of tandem transplantation, therefore second myeloablative treatment was reserved only for patients who did not achieved good remission (reduction of M-component < 75%) during 6 months after transplantation. After 5 years the data is still not mature enough and our expectation of advantage of tandem transplantation probably will not be fulfilled as the patients with MM after tandem transplantation frequently relapsed (4, 11). Multiple myeloma is thought to be incurable by all treatment modalities including autologous transplantation, but a small minority @ 5 % of MM patients treated with myeloablative regimens do not relapse 10 years or longer (16). This fact should also be evaluated in other trials using autologous as well as allogeneic transplantation.

In the trial 4W, very routine induction (VAD), mobilization (CFA+G-CSF) and myeloablative regimen (melphalan 200mg/m^2) have been used. Evaluations of toxicity of mobilization regimen as well as myeloablative regimen are comparable with toxicity data reported in similar trials (9, 10). Transplant related mortality (3.1 %) is also acceptable and comparable with other trials (9, 10). We have not been able to confirm a high rate of CR after transplantation as reported in some other trials (4). But a 5 % increment of CR from 7 % CR (8 patients) after VAD to 12 % CR (13 patients) 1 month after transplantation is in fact an improvement of 63 %. It should be mentioned that a small number of patients with undetectable M-component (9 patients) did not have immunofixation due to several reasons and their response was strictly evaluated as remission (R) and not CR. But even though some of these patients would fulfill the criteria for CR, the absolute number of CR would remain low in this trial. Probably incomparable data are

compared across different trials as complete remission was defined in different ways, especially in older trials (28) and some trials did not require negative immunofixation (9). Interim analysis has shown a high proportion of patients achieving OR (CR+R+PR) after VAD (83 %) and 1 month after transplantation (89 %). The number of non-responders (SD+PG) after VAD (10%) and transplantation (4 %) was very low and only a small proportion of patients (8.9 %) had achieved less response than R (<75%) evaluated 6 months after transplantation and required second transplantation according to clinical protocol 4W.

The main interest in this trial is focused on maintenance therapy after transplantation in which standard dose of IFN (3 x 10^6 units three times weekly) has been compared with IFN and dexamethasone in randomized fashion. Despite many trials undertaken, no firm conclusion can be drawn about the role of IFN in the treatment of myeloma as mentioned above. Only the data from retrospective analyses associate IFN maintenance therapy with better prognosis in patients with MM after autologous transplantation (5). The first randomized trial has failed to prove the advantage of IFN maintenance therapy after transplantation (6). Interferon may be more effective in the setting of minimal residual disease (15), although we are not able to identify patients who will benefit from IFN maintenance therapy yet. Corticosteroids have been added to maintenance therapy as one of the most effective drug in multiple myeloma (1). Data from randomized trial of maintenance therapy of IFN plus prednisolone after conventional treatment has shown the advantage of combined maintenance therapy against IFN (34). Preliminary analysis of a similar trial conducted by Ludwig et al. using lower dose of prednisolone (25mg 3 x weekly vs. 50mg every other day) has not shown any differences (Ludwig, personal communication). Recently published results of randomized trial of IFN (3x 10^6 units three times weekly) versus dexamethasone (20mg for 4 days every month) after primary therapy with oral melphalan and dexamethasone have shown identical duration of median remissions of 10 months with IFN or dexamethasone (35). Significantly more patients responded again to resumption of melphalan and dexamethasone after disease relapse to IFN (82%) than to dexamethasone (44 %, p=0.001). Similar data after myeloablative regimen are not available.

Economic aspects and quality of life concerning the maintenance therapy should be taken into account. The reasons for the sequential schedule in arm IFN/DEX were to evaluate the benefit of dexamethasone addition to IFN on EFS and OS, as well as the benefit of treatment free period (six months without IFN therapy and four moths without any therapy) for quality of life. Data of quality of life and toxicity of IFN and IFN+DEX are still pending and preliminary results will be included in a special in-coming analysis focused on toxicity and quality of life of different maintenance therapy. Interestingly, a smaller proportion of about 4 % patients would preferred dexamethasone only as optimal maintenance therapy, IFN was preferred in 12 % of patients and one third of patients confirmed sequential combination IFN/DEX as optimal maintenance therapy.

Optimal maintenance therapy is not known and new approaches like intensive consolidation therapy (36), thalidomide (37) or vaccination using dendritic cells (38) are potentially beneficial and should be evaluated in randomized trials. In this trial, despite the fact that the data are not mature, no significant differences have been found after randomization of the first 100 patients, although a higher

number of relapsed patients in the IFN/DEX group (23 vs. 13) was shown. The results are still preliminary and longer follow-up is required to come to some conclusions about comparison between IFN and IFN+DEX as maintenance therapy in MM patients. The high response rate and acceptable toxicity of autologous transplantation have been confirmed. Differences between randomized groups of patients are nonsignificant. More patients and more follow-up period are needed for final conclusions.

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Table 1. Clinical trial 4W - patients characteristics

		IFN	IFN/DEX	P Value
Number of patients		54	59	
Female		18	19	
Male		36	40	
IgA		19%	25%	
IgG		65%	61%	
IgD		1%	2%	
Bence-Jones		15%	12%	
Stratifying criteria:				
Age				0.979
	Mean	52.4	52.5	
	Range	33-64	32-67	
Stage				0.628
	I	4	7	
	II	13	16	
	III	37	36	
Response after induction (4x VAD)				0.851
	≥75% R	30	31	
	<75% R	24	28	

Abbreviations: IFN, Interferon treatment arm; IFN/DEX, Interferon+Dexamethasone treatment arm; VAD, vincristine, doxorubicin, dexamethasone; R, reduction of M-component.

Table 2. Response to induction treatment and myeloablative

treatment No. of 113 patients	after 4xVAD	1 month after transplantation
Complete Remission	**8** (7%)	**13** (12%)
Remission	**54** (48%)	**63** (56%)
Partial Remission	**32** (28%)	**24** (21%)
Minimal Response	**8** (7%)	**8** (7%)
Stable disease	**11** (10%)	**4** (3%)
Progression/Relapse	**0** (0%)	**1** (1%)

Table 3. Comparison of potential predictors in randomized arms

Parameter	N	IFN Median (IQ range)	IFN/DEX Median (IQ range)	P Value
CRP (mg/l)	100	3.9 (0.0-7.9)	6.3 (1.0-16.0)	0.026
β 2 microglobulin (mg/l)	99	3.6 (2.7-5.7)	3.4 (2.6-5.0)	0.571
Albumin before treatment (g/l)	111	37.0 (32.0-41.4)	38.2 (32.0-42.0)	0.781
Albumin before PBSCT (g/l)	106	41.0 (34.0-45.0)	40.0 (34.1-43.5)	0.667
LDH before treatment (μkat/l)	104	5.3 (4.2-6.2)	4.8 (4.0-6.3)	0.433
LDH before PBSCT (μkat/l)	105	6.3 (5.3-7.5)	6.2 (5.2-8.0)	0.820
Platelets count (10^9/l)	113	219 (190-282)	226 (166-275)	0.600
Haemoglobin (g/l)	113	104 (91-125)	109 (92-120)	0.881

Abbreviations: IFN, Interferon treatment arm; IFN/DEX, Interferon+Dexamethasone treatment arm; PBSCT, peripheral blood stem cells transplantation

Table 4. Toxicity of mobilization chemotherapy (SWOG)

Type/Grade	0	1	2	3	4
Renal toxicity	96	11	2	1	0
Toxicity of GIT	77	23	10	1	0
Hepatotoxicity	94	15	1	1	0
Mucositis	86	18	4	1	2

Table 5. Toxicity of myeloablative chemotherapy (SWOG)

Type/Grade	0	1	2	3	4
Renal toxicity	99	8	4	0	1
Toxicity of GIT	32	30	31	15	4
Hepatotoxicity	92	8	12	0	0
Mucositis	36	28	19	14	15

Figure 1. Trial 4W schedule

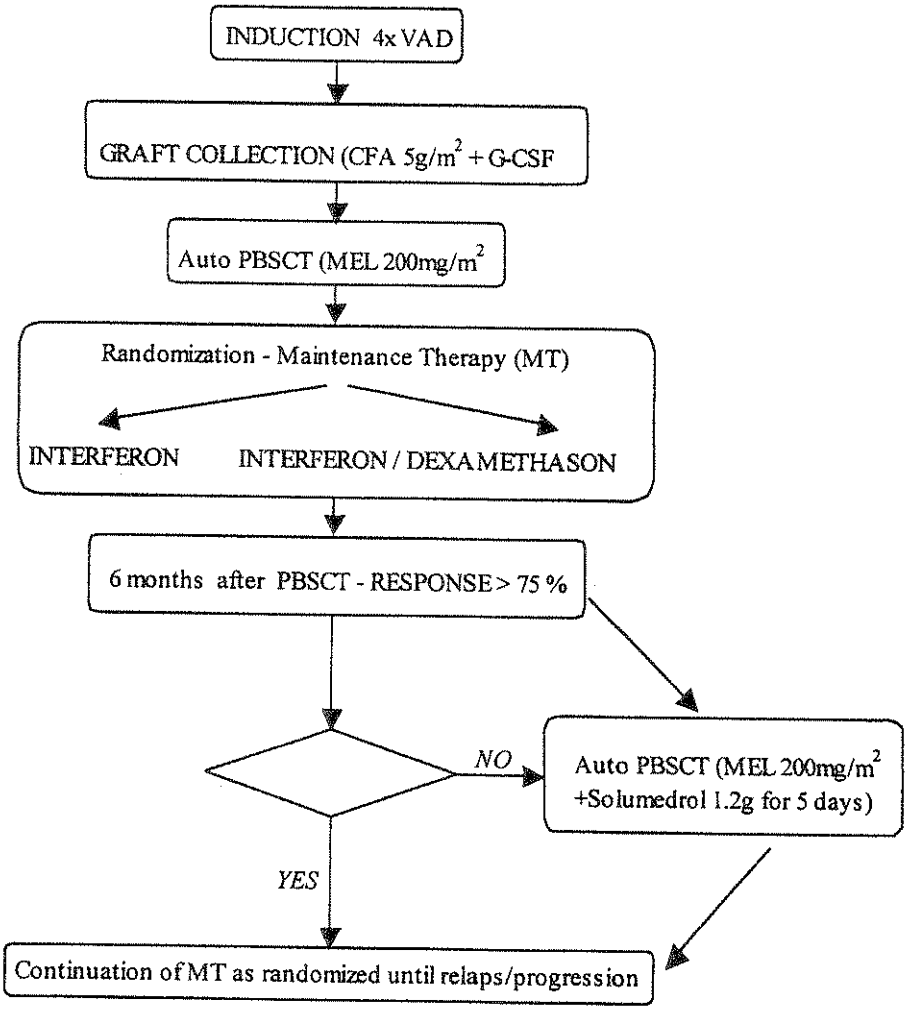


Figure 1. Trial 4W schedule.
VAD = vincristine, doxorubicin, dexamethasone; CFA = Cyclofosfamid; G-CSF = Granulocyte Colony-Stimulating Factor; PBSCT= peripheral blood stem cell transplantation; MEL = Melphalan

Figure 2. Event free survival (EFS) and overall survival (OS)in all randomized patients

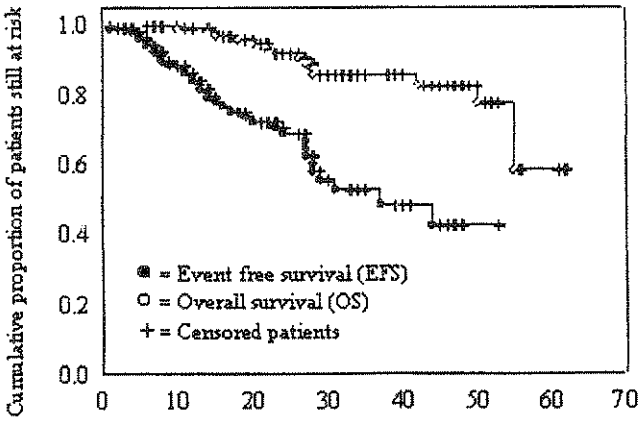


Figure 3. Event free survival (EFS) in the two treatment arms

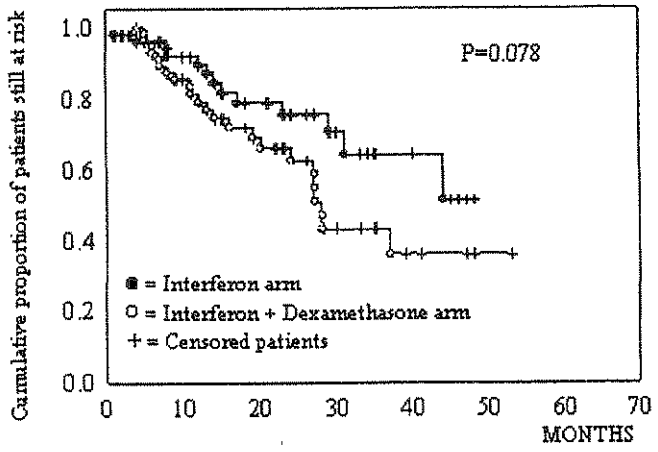
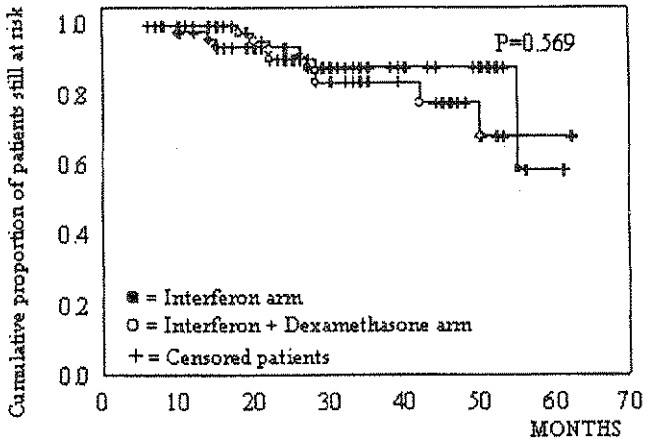


Figure 4. Overall survival (OS) in the two treatment arms



REFRACTORY ASCITES AND SPONTANEOUS BACTERIAL PERITONITIS

Jan Lata, Petr Dítě

Department of Internal Medicine and Gastroenterology
University Hospital Brno, Czech Republic

Address correspondence to:

Prof.MUDr.Petr Dítě,DrSc.
Head
Department of Internal Gastroenterology
Faculty of Medicine, Masaryk University
Jihlavská 20
639 00 Brno

Ascites is defined as an accumulation of fluid in the peritoneal cavity - the normal amount of fluid is approximately 150 ml - and is a common complication of liver cirrhosis (even it can be also found in some other diseases, eg. TBC infection, carcinomatosis of the peritoneum, pancreatitis etc.) Ascites can be easily detected by ultrasound examination. For detection on physical examination approximately 1,5-3,0 l of fluid are needed. Appearance of ascites is always a bad prognostic sign and the 1-year survival of patients who are admitted to the hospital because of ascites is about 56%. Three theories have been advanced to explain sodium and water retention in the cirrhotic patient with ascites: on the basis of the underfill theory, increased hepatic resistance to portal blood flow combined with low serum oncotic pressure causes the extravasation of fluid into the peritoneal cavity. It leads to decrease of the effective arterial blood volume which results in sodium and water retention by kidneys (29). Retention of sodium on the other hand is found even in patients without ascites or this retention precedes the formation of ascites. So the overfill theory was proposed - inappropriate primary renal tubular retention of sodium increases plasma volume causing an increase in the plasma compartment and in the presence of portal hypertension results in translocation of fluid as ascites. In this theory, there is no clear explanation why the kidneys first start to retain sodium.

The third theory - peripheral arterial vasodilatation - was proposed and has gained wide acceptance because it explains most of the hemodynamic changes present in cirrhosis (27). The first step seems to be an increasing of portosystemic collaterals (intra and extrahepatic) that leads to increasing levels of many vasodilators (glucagon, VIP, endotoxins etc) in the systemic circulation. These agents stimulate production of endothelial vasodilatator agents (prostacyclin, EDRF, NO) (28). Peripheral arterial vasodilatation (primarily in the splanchnic bed) causes a decrease in the effective circulating blood volume leading to the reabsorption of sodium and water by kidneys.

Ascites is usually treated by dietary sodium restriction and diuretics (spironolactone and furosemide), but 10 - 20% of patients do not respond to this therapy or this treatment has to be stopped because of complications (3). This kind of ascites is called refractory.

DEFINITION

A revised definition of refractory ascites was proposed in a Consensus Conference organized by the International Ascites Club (1). According to this new proposal, refractory ascites is ascites that cannot be mobilized or the early recurrence of which (i.e., after therapeutic paracentesis) cannot be satisfactorily prevented by medical therapy. The term refractory ascites includes two different subtypes: "diuretic-resistant ascites" and "diuretic-intractable ascites".

"Diuretic-resistant ascites" cannot be mobilized or the early recurrence of which cannot be prevented because of a lack of response to dietary sodium restriction and intensive diuretic treatment.

"Diuretic-intractable ascites" cannot be mobilized or the early recurrence of which cannot be prevented because of the development of diuretic-induced complications that preclude the use of an effective diuretic dosage.

Refractory ascites is often associated with mild renal insufficiency. Survival of patients with refractory ascites is very low - more than 50% of patients die within 2 years after the onset of this condition. The treatment usually can control refractory ascites, but poor survival rate is observed after all kind of therapy. As refractory ascites is often a terminal event in the course of cirrhosis, liver transplantation should be considered in these patients if possible.

PARACENTESIS

Paracentesis has been used as a treatment of ascites for centuries but the safety of this procedures was widely discussed not far in the past. Renal failure, cardiovascular collapse and even death after paracentesis were described in the literature. These major complications were rare, but many other minor complications were present - symptomatic hyponatremia, protein depletion and especially circulatory changes (14), which deteriorated already damaged circulation in patients with advanced liver disease. The post-paracentesis circulatory dysfunction is characterised by marked activation of vasoconstrictor and antinatriuretic systems without a reduction in plasma volume, changes consistent with an impairment of the effective blood volume (19). This disturbance in the systemic circulation after paracentesis is most likely due to a further vasodilatation of the arterial vascular bed of cirrhotic patients. Once it has developed, post-paracentesis circulatory dysfunction is not spontaneously reversible and has a negative impact on the evolution of the disease, because it has been shown that the development of this abnormality is associated with higher incidence of ascites reaccumulation and increased morbidity (mainly renal impairment and hyponatremia) in patients treated with paracentesis without plasma expanders (8). This procedures has been contraindicated except for diagnosis and for the urgent relief of tense ascites (23) till 1985, when therapeutic paracentesis with plasma expanders was reintroduced for the treatment of ascites (24).

During the last decades, the results of several randomized studies showed that paracentesis with plasma volume expansion (that protects the patient from above mentioned circulatory changes) is a safe and very effective treatment of refractory ascites. Infusion of i.v. albumin decreases possibility of clinical or subclinical hypovolemia and deterioration of renal function and even total paracentesis is a safe procedure (31). The amount of albumin needed for replacement after paracentesis is at least 6 - 8 g/l of evacuated ascitic fluid. The total amount of albumin can be injected immediately after paracentesis or it can be divided into two parts: 50% of albumin is given immediately after and 50% 6 hours later, or 50% of albumin is given 6 hours and 50% 12 hours after the paracentesis.

This treatment is safe, but expensive. For that reason, cheaper plasma expanders have been looked for. Heamacel or Dextran 40 or 70 can be used with the amount used being equal to albumin replacement. These artificial plasma expanders have similar efficacy as albumin when less than 4 l of ascites is removed. However, when more than 4 l of ascitic fluid is removed, albumin is more effective than other plasma expanders (10).

If paracentesis is performed, large-volume (LVP) or total paracentesis (TP) is recommended. LVP is defined as removal of 4-6 l/day and usually is performed daily until the ascites is completely mobilized. TP consists of removal of ascitic fluid in one setting. TP (up to 31 L of ascitic fluid can be safely removed in one session) is as effective as repeated LVP, does not increase mortality or morbidity and shortens hospital stay.

Patients treated by paracentesis should receive diuretics immediately after this procedure to prevent early recurrence of ascites. The administration of 200 mg/day of spironolactone is recommended for non-azotemic patients. This dose is effective in most cases and does not increase the incidence of post-paracentesis circulatory dysfunction (5).

Paracentesis with albumin replacement is the most commonly used method in the treatment of refractory ascites. However, when the need for paracentesis becomes more frequent than monthly, an alternate form of therapy needs to be considered.

TRANSJUGULAR INTRAHEPATIC PORTOSYSTEMIC SHUNT

Since its inception in clinical practice, the role of transjugular intrahepatic portosystemic shunt (TIPS) in the management of patients with chronic liver disease has been expanding. TIPS was initially used to treat patients with refractory variceal bleeding and later to treat even acute bleeding. Decrease of portal pressure and observation that many patients with ascites had marked improvement in ascites after TIPS led to studies evaluating the role of TIPS as a treatment of refractory ascites.

TIPS was first used in this indication in the early 1990's (6,17) and the usefulness of TIPS was evaluated in several studies (13,20,21). This procedure is associated with a marked suppression of renin, aldosterone and norepinephrine and an improvement in renal function and renal response to diuretics. TIPS is a safe procedure and allows good control of ascites in nearly 70% of the cases. This procedure can even improve overall clinical status - increase in lean body mass, improvement in Child-Pugh score (32).

Patients, with pre-TIPS Child-Pugh score higher than 11 do not respond and have poor survival rate. The onset of post-TIPS accelerated liver failure has been described in some patients but is not very frequent. Hemodynamically, TIPS works as a side-to-side portacaval shunt so the incidence of new or worsened hepatic encephalopathy is 17 - 25%, mostly in patients older than 65 years. The other disadvantage of this safe procedure is frequent obstruction of the shunt that has been described in up to 30% of cases and repeated interventions such as stent dilatation or addition of new stents are frequently required (26).

TIPS may serve as a bridge to transplantation and improved nutritional status may decrease need for transplantation in some patients. Ideal candidate for TIPS seems to be patient without previous history of hepatic encephalopathy with Child-Pugh score less then 11, in whom quality of life is severely impaired by repeated paracentesis.

In patients who are not candidates for liver transplantation, the placement of TIPS is more controversial but usually is preferred to peritoneovenous shunting or surgical shunts.

PERITONEOVENOUS SHUNTING

Peritoneovenous shunt connectings peritoneal cavity with central vein was introduced by LeVeen in 1974 (18). Numerous studies showed that peritoneovenous shunting is associated with marked suppresion of renin, aldosterone, norepinephrin and antidiuretic hormone, indicating an improvement of circulatory dysfunction, an amelioration of renal function and an increased response to diuretic treatment (2).

On the other hand, the use of this procedure in clinical practice has not been so successful. A lot of early (acute bacterial infection, subclinic disseminated intravascular coagulation) and especially late complications were described. In 30 % of cases shunt obstruction appears and even Denver shunt with a valve, which was specifically designed to prevent this complication, was not effective in reducing this problem (7). In about 10 % obstruction of small intestine could be found as a result of intraperitoneal fibrosis. Patients with ascites infection, congestive heart failure and severe coagulopathy should not be offered these shunts.

Therapeutic paracentesis has been shown to be as effective as peritoneovenous shunting and the number of complication, mortality, hospitalisation stay and survival do not differ (11).

Peritoneovenous shunt is usually reserved for patients with refractory ascites suffering repeated paracentesis and, more often, for those who are poor candidates for transplantation or TIPS.

PORTAL-SYSTEMIC SHUNT SURGERY

The first surgical portocaval shunt as a treatment of ascites was used in 1903 (33) and since that time, portocaval shunting has been known as an effective means of relieving refractory ascites. Side-to-side portocaval shunts are thought to be more effective than end-to-side shunts because they provide a reversed-flow outflow tract for hepatic artery blood via the portal vein. Surgical mortality was up to 40% in older studies; in the more recent studies it is less than 5% (4). The

incidence of worsening encephalopathy varies, although a rate of 30% has be seen, similar to TIPS procedure. Selective shunts which decrease the incidennce of post-shunt encephalopathy may worsen ascites in some patients.

Long-term survival and increased quality of life may be achieved in some patients undergoing side-to-side portocaval shunt for refractory ascites (22).

Portal-systemic shunt surgery is usually an alternative to TIPS procedure in some cases. On the other hand, the recent study, evaluating the number of procedures, life expectancy and costs over the first 2 years in patients with Child-Pugh class Acirrhosis who uderwent a TIPS or distal splenorenal shunt shows surgury as a more cost-effective treatment than TIPS (34).

REINFUSION OF CONCENTRATED ASCITES

Concentration and reinfusion of ascites can be used as alternative therapy. It was first proposed and in some cases used in the end of the 1950's as a logical step in removing just water from the body. Wider clinical use of this therapy was achieved in the 1970's using Rhodiascit machine (23). Several methods of ascites concentration (spontaneous filtration, reverse cascade filtration, apheresis and mostly dialytic ultrafiltration using hemodialysis machines) and reinfusion (intravenouslly or intraperitoneally) have been used. Recently several studies were performed using this technique as alternative methods.

Apheresis and intravenous reinfusion of concentrated ascites (with removal of up to 10 L of ascites) is safe and as effective as paracentesis with albumin replacement and has advantage in savings patients proteins (12).

Intraperitoneal reinfusion can be performed safely as well. There could be an advantage in the prevention of spontaneous bacterial peritonitis, which is a very frequent and dangerous complication of ascites. We have shown, that this therapy can influenced spontaneous bacterial peritonitis - reinfusion increases amount of antibodies and C3 and C4 complement in ascites, the deficit of which seems to play an important role in the pathogenesis of spontaneous bacterial peritonitis (16).

SPONTANEOUS BACTERIAL PERITONITIS

Bacterial infections are generally frequent and severe complications of liver cirrhosis. Spontaneous bacterial peritonitis (SBP) is the most frequent one and is defined as bacterial infection of ascitic fluid without any apparent intra-abdominal focus. Its prevalence in cirrhotic patients with ascites admitted to a hospital range between 10 - 30%. We have shown, that prevalence in the Czech Republic is even higher - 35,5% (15). SBP is severe complication with mortality rate up to 80% in older studies, in spite of progress in the therapy the mortality rate in recent studies is still up to 40% (30). Rate of recurrence is very high as well - approximately 70% per year.

SBP is diagnosed by increasing of polymorphonuclear leukocytes (PMN) in ascites over 250/mm3 or leukocytes over 500/mm3. Ascitic fluid culture is usually negative, so despite negative ascitic fluid culture, patients with increased ascites PMN count should be considered as having SBP. Symptoms are usually unimpressive and diagnostic paracentesis has to be performed in all cirrhotic patients with ascites admitted to hospital.

Bacterial translocation (passage of bacteria from the intestinal lumen to regional lymph nodes and/or the systemic circulation) and decrease of opsonic activity of ascites are considered to be important factors in the development of SBP.

Antibiotic therapy must be initiated in patients with an ascitic PMN count > 250 mm3 (25). Several antibiotics can be used for the initial empirical therapy with similar efficacy: cefotaxime, other cephalosporins (cefonicid, ceftriaxone, ceftizoxime, ceftazidime) or amoxicilin-clavulanic acid. The optimal regime seems to be cefotaxime 2 g/ 12 hours and a minimum duration of 5 days of therapy is recommended. The other possibility for uncomplicated SBP is ofloxacin, minimum dose of 400 mg/12 h. Addition of intravenous albumin to an antibiotic seems to reduce the incidence of renal impairement and death of these patients (9). The response to treatment should be assessed by periodically evaluating the symptoms and signs of infection and at least one follow-up paracentesis after 2 days of therapy.

As a prophylaxis, oral administration of norfloxacin (400 mg/12 h) is recommended in patients with upper gastrointestinal bleeding over a minimum period of 7 days. The same treatment is recommended for patients recovering from an episode of SBP. Since survival probability is very much reduced after SBP, cirrhotic patients who have recovered from and episode of SBP should be evaluated for liver transplantation. There is no consensus for the prophylactic therapy in patients with low ascitic fluid protein concentration (< 10 g/l) and low opsonic activity of ascites, in spite of knowledge that these patients has SBP in high percentage (25). As mentioned above already - we have shown in the study in 26 patients that intraperitoneal reinfusion of concentrated ascites significantly increases opsonic activity of ascites and could be in these patients useful in the prevention of the development of SBP (14).

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EJECTION FRACTION AND BAROREFLEX SENSITIVITY IN PATIENTS AFTER MYOCARDIAL INFARCTION

Semrád Bořivoj[1], Honzíková Nataša[2], Fišer Bohumil[2]

[1]First Department, Faculty of Medicine and [2]Department of Physiology, Masaryk University, Brno, Czech Republic

Address correspondence to:

Prof.MUDr.Bořivoj Semrád,CSc.
Head
Department of Internal Cardiology Medicine
Faculty of Medicine, Masaryk University
Jihlavská 20
639 00 Brno

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ABSTRACT

Baroreflex sensitivity expressed in ms/mmHg (BRS) is correlated with the pulse interval (PI) in healthy subjects but is PI-independent when expressed in Hz/mmHg (BRSf). The relationship between the ejection fraction (EF) and BRS and BRSf values in patients after myocardial infarction (MI) was the aim of the present study.

BRS and BRSf were determined by spectral analysis of PI and systolic blood pressure fluctuation in 117 patients, 7 to 14 days after the first signs of MI. EF was determined by echocardiography.

At EF between 40-70% (n=92), BRS and BRSf remained constant (mean±SD: 5.5±3.38 ms/mmHg, 0.00779±0.00511 Hz/mmHg). At EF 30-40% (n=17), lower values of BRS and BRSf were observed (BRS: 58.1±48.5%, BRSf: 67.5±54.7% of the values at high EF). The difference between BRS and BRSf was significant ($p<0.05$). At EF 20-30% (n=8), the values of BRS and BRSf were even lower (BRS: 33.9±23.6%, BRSf: 34.9±21.3% of the values at high EF).

The results show that the values of the PI-dependent BRS correspond to the baroreceptor sensitivity altered at EF below 40%. Therefore, it can be concluded that the activity of the autonomic nervous system was altered, too. The PI-independent BRSf corresponds only to the baroreceptor sensitivity decreased at very low EF, probably due to an aldosterone influence.

INTRODUCTION

After myocardial infarction (MI) patients are at risk of cardiac death (CD). Prediction of the risk of CD is currently important, because implantation of a cardioverter may provide protection against it. Many indices of cardiac and circulatory functions have been investigated to determine their predictive role. Many studies, and especially the multicentre ATRAMI study(1), have shown that increased sympathetic and/or decreased parasympathetic autonomic nervous system activity (indicated by a decrease in baroreflex sensitivity, BRS) and decreased contractility (indicated by a decrease in the ejection fraction, EF) are two of the three most important independent predictors of the risk.

In most studies, baroreflex sensitivity is expressed in ms/mmHg, and this index is correlated with the pulse interval (PI) in both healthy subjects and in patients after MI (2). BRS mainly reflects the heart rate baroreflex component, including parasympathetic and sympathetic tonic nervous activity, and the sensitivity of receptors.

Baroreflex sensitivity, when expressed in Hz/mmHg (BRSf), is PI independent (3) but, in children, it decreases with age, as does the compliance of arteries (4). BRSf correlates with the second value obtained by repeated investigation after a 1-year interval, as was shown by the examination of 88 young adults (4). It seems that BRSf corresponds more to the sensitivity of baroreceptors than to a central gain of the baroreflex. In patients after MI, both the activity of the parasympathetic autonomic nervous system and the sensitivity of baroreceptors may be impaired.

In many patients after MI, the number of risk factors increases and, therefore, the overall risk to patients is increased. Decreased contractility, indicated by a decrease in the ejection fraction to under 40% (5), is one of these risk factors. In heart failure, not only an increased sympathetic activity may be present, but also a decreased excitability of the baroreceptors. It has been demonstrated in dogs with experimental heart failure that the depressed carotid sinus baroreceptor reflex is a result of depressed baroreceptor responsiveness and of a poor end-organ response (6). The aim of the present study was to establish the relationships between EF and BRS and EF and BRSf in patients after myocardial infarction.

MATERIALS AND METHODS

We studied 117 patients (56.8±9.0 years old) 7 to14 days after the first signs of myocardial infarction. The diagnosis of acute myocardial infarction was based on conventional clinical, electrographic and enzymatic criteria.

Indirect, continuous blood pressure recordings from finger arteries (Finapres, Ohmeda), lasting for 3 min, were performed in sitting and resting patients between 9 a.m. and noon. Recordings were taken during spontaneous and synchronised breathing. During the latter, the rhythm of breathing was controlled by metronome (0.33 Hz; 20 breaths per min) but the subjects were allowed to adjust the tidal volume according to their own comfort. Beat-to-beat values of systolic pressure and pulse intervals were measured for further analysis. For spectral analysis, the parameters were linearly interpolated and equidistantly sampled at 2 Hz. The linear trend was removed. The auto-correlation and cross-correlation functions, power spectra and cross-spectra, coherence and the modulus between pulse intervals and systolic pressure were calculated. The gain factor,

e.g., modulus of the transfer function between variations in systolic blood pressure and PI at a frequency of 0.1 Hz, was taken as the index of BRS (ms/mmHg)(7). The values of BRS were taken into account only if the coherence between systolic blood pressure and PI at 0.1 Hz was higher than 0.5 Hz. Sensitivity of the baroreflex was also expressed in Hz/mmHg as BRSf (8).

EF was determined by a two-dimensional echocardiogram (Accuson 128 XP/10). The patients were separated into 5 groups according to EF (EF value, number of patients: group 1, 20-29%, 8; group 2, 30-39%, 17; group 3, 40-49%, 37; group 4, 50-59%, 38; group 5, >60%, 17). The difference between BRS and BRSf in each group was tested by the Wilcoxon test for paired values.

RESULTS

The values (mean± SD) of BRS and BRSf calculated for the five groups are presented in *Table 1*. At EF of 40-70% (groups 3 to 5, n=92), BRS and BRSf remained constant (5.5±3.38 ms/mmHg and 0.00779±0.00511 Hz/mmHg). These values were taken as 100% and compared with those in lower EF ranges (groups 1 and 2) and the relative decrease in BRS and BRSf values was calculated. At EF of 30-40% (group 2, n=17), lower values of BRS and BRSf were observed (58.1±48.5% and 67.5±54.7% of the values at high EF). The difference between BRS and BRSf was significant ($P<0.05$). At EF of 20-30% (group 1, n=8), the values of BRS and BRSf were even lower (33.9±23.6 % and 34.9±21.3% of the values at high EF). These results are illustrated in *Fig. 1*.

DISCUSSION

Our results support the hypothesis that the value of baroreflex sensitivity is not a fully independent factor in patients after MI; it responds to a decrease in EF value lower than 40%. The relative decrease of BRS at EF below 40% might have indicated the primarily altered heart rate baroreflex component of the autonomic nervous system activity. Our results are in agreement with the experiments in dogs. Depressed responsiveness of the sinus node to an increase in pressure and to cholinergic stimuli was proven in heart failure experimentaly induced in dogs (9). Ouabain administration increases the sensitivity of the carotid sinus node in heart failure in dogs (10). It can be speculated that, in our patients, there was an increased activity of aldosterone, which is present in heart failure. Aldosterone diminishes the baroreceptor's sensitivity by activation of Na^+-K^+ ATPase. Therefore, the effect of aldosterone can also be involved in the decrease in baroreflex sensitivity found in our study. It was also shown in dogs that a depressed baroreceptor reflex in heart failure was not solely the result of depressed baroreceptor responsiveness but might have been related to poor end-organ responses (6).

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Fig. 1: Relationship between the ejection fraction (EF) and two indices of baroreflex sensitivity, BRS and BRSf, expressed in %. The mean values of BRS and BRSf at EF in the range of 40-70% are taken as 100%.

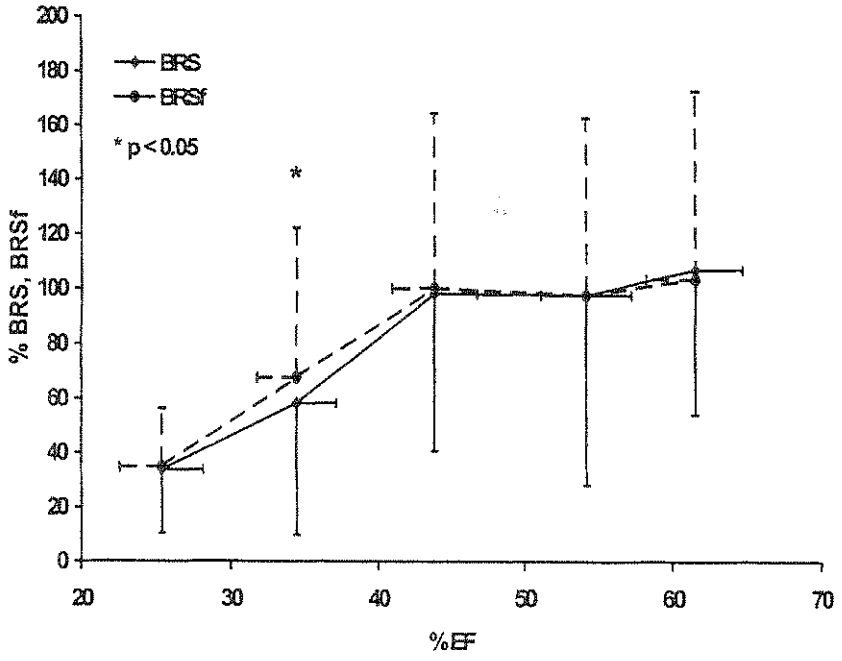

Table 1. Groups of patients according to the ejection fraction (EF) and the corresponding baroreflex sensitivity values expressed in ms / mmHg as BRS and in Hz / mmHg as BRSf.

EF mean±SD [%]	BRS mean±SD [ms/mmHg]	BRSf mean±SD [Hz/mmHg]
25.4±2.8	1.9±1.3	0.0027±0.0017
34.5±2.7	3.1±2.6	0.0053±0.0043
43.9±2.9	5.4±3.1	0.0078±0.0050
54.2±3.1	5.4±3.8	0.0076±0.0051
61.5±3.2	5.9±2.9	0.0081±0.0054

COMMON WAYS OF CARDIOVASCULAR DISEASES

Jiří Toman

First Department of Internal Medicine - Cardioangiology
Faculty of Medicine, Masaryk University

Address correspondence to:

Prof.MUDr. Jiří Toman,CSc.
Head
First Department of Internal Medicine - Cardioangiology
Faculty of Medicine, Masaryk University
Pekařská 53
656 91 Brno

Over the last years research has increasingly showed that a range of cardiovascular diseases and conditions that significantly attack population have many common pathophysiological basis and mechanisms. Among these diseases and conditions especially belongs **atherosclerosis** with its clinical signs (above all **ischaemic heart disease, cerebrovascular disease, occlusion arterial disease of lower extremities), hypertension, diabetes mellitus, chronic heart failure, ageing.** There is increasing evidence that ways of progression of these diseases partially overlap or fade one into another. Mechanisms, which are efficient elements, take part in various situations in a similar or the same way, though possibly in a different phase of the process. Another common features are **risk factors** and **ways of transferring signals**.

These principles have probably developed during the long-term phylogenesis in reaction to various insultuses from both the external and internal environments. They are also relatively fixed, so that in some situations are improper and have adverse effects, are maldaptivní, such as chronic heart failure. Irreversible myocardial dysfunction isn't unknown even in lower animal species. In a man (independently of aetiology) initiates complex regulations (a leading role has the neurohumoral activation), originally intended for managing other situations, especially to managing lost of circulatory volume (bleeding). Improper situations worsen basic disorder, lead to positive feedbacks and vicious circles which, when not interrupted by treatment interventions, lead to uncontrollable heart failure and breakdown of homoeostasis.

Fortunately, increasing knowledge also led to intensive search for therapeutical practices which try to hinder adverse progress. Resulting in both pharmacological and nonpharmacological principles of treatment which are often common for solving these pathological conditions too.

MAIN PATHOPHYSIOLOGICAL COMPLEXES (SYNDROMES)

Endothelial dysfunction

Endothelium has four main functions. It controls blood flow in vessels by changed tonus of smooth musculature. Functionally efficient endothelium

supports vascular vasodilatation and hinders its vasoconstriction. Endothelium also controls permeability of both cellular and noncellular component of blood. Its another function is the thrombogenic control. It hinders adhesion and aggregation of thrombocytes and keeps frail balance between activation of coagulation system and inhibition of fybrinolysis. The key mechanism by which endothelium influences the tone of vessels is secretion of *nitrogen oxide* (NO). Nitrogen oxide is formed from L-arginine in endothelium and initiates venous dilatation.

Undamaged endothelium produces range of agents of which many have antagonistic function. Functional or anatomical change of the vessel depends on their proportion. Vasodilatation is caused by production of NO, prostacyclin, bradykinin, acetylcholine, and endothelium-derived hyperpolarizing factor. On contrary, vasoconstriction is supported by endothelium-derived contracting factors (EDCF). Primary contracting factor is endothelin 1. Its secretion is stimulated by the hypoxia, cytokines, angiotensin II, adrenaline, thrombin, and LDL-cholesterol. Secondary contractile factors are thromboxane A2 and angiotensin II.

The some equilibrium is kept between agents affecting readiness for thrombosis and inflammation. The activation of blood platelets affects NO, PGI2, von Willebrand´s factor, tissue activator of plasminogen and thrombomodulin. Antiinflammatory factors generally counteract genesis of atherosclerosis and vice versa. Equilibrium of proliferation and migration control in venous wall and control of oxidative and antioxidative actions is also important.

The equilibrium of endothelial function is above all impaired by risk factors. The most important risk factors are **genetic predisposition, sex, age, hypercholesterolemia, smoking, hypertension, obesity, diabetes mellitus, highprotein diet**. Endothelial dysfunction and linked atherogenesis can already start in childhood. In children of smoking parents who are exposed to cigarette smoke develop characteristic "fatty strips" in the venous wall. In following age decades we can find more advanced characteristic changes. There cumulate foamy cells, smooth musculature penetrates developing plate, and develops fibrous matrix stabilising plate, in the venous wall. Atherosclerotic plates grow and cause symptoms of ischaemia in coronary, brain, or peripheral vessels. Follows dissolving of protection fibrous "cap" of the plate by metalloproteinases resulting in atherothrombosis, plate rupture and arterial occlusion via thrombus.

Remodeling

Chronic haemodynamic overload together with other pathogenetic mechanisms lead to structural reconstruction both in heart and in vessels in which participate all the components of the tissue. By increased ventricular volume and by extension of the relation pressure-volume this remodelling makes possible managing pulse volume by the heart, even in presence of impaired contractility. The remodelled heart is not only bigger but it is of more spheric shape. High end-diastolic stress can lead to episodes of hypoperfusion of subendocardial layers with further worsening of function of the left ventricle. Increased wall stress can also lead to gene expression activated by increased tension in ventricle (angiotensin II, endotelin, and tumour necrosis factor alpha).

An important part of remodeling is hypertrophy. Many determinants of left ventricle hypertrophy have already been detected. Validated include: increased blood pressure/wall stress, pulse volume, obesity, growth hormone, male sex, race,

age, increased amount of intracellular calcium, insulin resistance, angiotensin II, aldosterone. Unconfirmed include: alcohol, myocardial contractility, increased blood viscosity, parathormone, increased sodium uptake, exchange systems, gene polymorphynism for angiotensin-converting enzyme (ACE), plasmatic renin activity, noradrenaline, ßARK.

Apoptosis

Apoptosis is genetically programmed cell death and is an important process in many cardiovascular diseases, including heart failure. In patients with chronic heart failure it affects 5-35% of myocytes in explanted tissue of heart muscle. Apoptosis can be initiated by hypoxia and increased activity of oxygen radicals in hypoxic cardiomyocytes. It is also affected by some cytokines, such as TFN alpha, interferon gamma, interleukin-1ß. Apoptosis is morphologically characterised by shrinked cell, condensation of nuclear chromatin, and by swelling of the cell organelles. Cell fragments into many apoptic corpuses which are absorbed by surrounding cells. Apoptosis is biochemically characterized by splitting of DNA into symmetric fragments.

MECHANISMS

Activation of humoral agents

Humoral systems participate in majority of processes. The key systems are renin-angiotensin-aldosterone system (RAAS) and sympathic-adrenal system (SAS). As already mentioned in the chapter concentrating on endothelial dysfunctions, very simplified division of humoral agents can be into vasoconstrictive and vasodilator agents. In pathological processes usually participates majority of vasoconstrictive agents which is from a viewpoint of a long-term development of an illness unfavourable. The agents of course have many other effects. A list of the most important humoral agents is presented in the table. The list of course is far from being complete and definite.

Vasoconstrictors	Vazodilators
Adrenaline	Prostaglandin E2,I2
Noradrenaline	Dopamine
Angiotensin II	Natriuretic peptides (ANP, BNP)
Aldosteron	Endothelial-derived relaxing factor.
Vazopresin	faktor (EDRF = NO)
Endothelin 1 (Big endothelin)	Adrenomedullin
Prostaglandin F2	Kallikrein-kinin system
Neuropeptide Y	Adenosine
Thromboxane A2	Vasoactive intestinál polypeptide
	Calcitonin gene-related peptide

Table: The most important humoral agents.

Pathological reactions are often initiated by haemodynamic changes, some of them provoke **shear stress**. An important role plays **oxidative stress**. A key role has NADH/NADPH oxidase system which is the main source of superoxides production in vennous tissue. Further, in many processes participates **inflammation**. There counterwork antiinflammatory cytokines (IL-6, IL-8, IL-1 beta, tumour necrosis factor alpha) in it. There is also growing evidence of participation of Chlamydia pneumoniae and other infectious agents. Adhesive molecules and adhesion of leucocytes are subjects of research. C-reactive protein is a good marker in this process. In **impaired coagulation** occur platelet dysfunction, decreased fibrinolysis, increased level of fibrinogen and other coagulopathy. Occurs change in **growth factors** expression. In many pathological situations occur **fibrosis, ischaemia and insulin resistance. Hyperhomocysteinemy** repeatedly proves to be important.

Evidence of **genetic stimulation** of pathological processes in cardiovascular system continuously growths. As an example we can use polymorphinism of genes for angiotensin-converting enzyme (ACE), genetically stimulated hyperfibrinogenemy or C242T polymorphinism p22 phox gene for NADH/NADPH oxidase.

TREATMENT AND PREVENTION

If we look at modern ways of treatment of above-mentioned cardiovascular diseases, we would find out that many remedies are active in more than one disease which supports the idea of common ways. The largest utilization have ACE inhibitors, ß-blockers and HMG-CoA inhibitors of reduktázy(statiny). Grows number of studies concentrated on an importance of interventions toward another humoral factors. The use of inhibitors of neutral endopeptidases, which hinder degradation of natriuretic peptides, seem to be promissing - results of OVERTURE study are awaited. Hopes laid on endothelin blockade have not fulfilled yet.

Disease progression can be also slowed down via non-pharmacological means, such as low fat diet, weight reduction, diet in diabetes mellitus, smoking abstinence. Proved is also influence of physical training (apart from other things on sympathoadrenal system and endothelial dysfunction).

Evidence concerning the importance of some remedies in secondary prevention increase as it is showed in results of extensive multicentral studies. The leading role have inhibitors ACE (SAVE, TRACE, AIRE, preventive branch SOLVD, HOPE, TREND), statiny (4S, CARE, AFCAPS/TexCAPS in primary prevention) and ß-blockers (CAPRICORN). The importance of acetylsalicylic acid has been known for a long time (Collaborative Overview of Randomized Trials of Antiplatelet Therapy). On the other side, the importance of antioxidation and estrogens has been still discussed.

As it results from present scientific knowledge, serious epidemiologically significant cardiovascular diseases have in many respects the some fundamentals and ways of progression. Some can be explained by cross interaction of vessels and heart. However, it is not very important whether the disease starts in vascular system and secondary affects heart or vice versa. At present we have non-pharmacological methods and remedies available with which we can intervene in the pathogenesis of these diseases, which is positive.

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HYPERTENSION - A RISK FACTOR OF CARDIOVASCULAR DISEASES

Jiří Zicha

Second Department of Internal Medicine,
Faculty of Medicine, Masaryk University

Address correspondence to:

Doc. MUDr. Jiří Zicha, CSc.
Head
Second Department of Internal Medicine,
Faculty of Medicine, Masaryk University
Pekařská 53
656 91 Brno

Arterial hypertension is one of the most important risk factors of cardiovascular diseases. High blood pressure is very closely related to diseases which have significant impact on the prognosis and life of the ill, especially if their basic disease isn´t under adequate control. Hypertrophy of the left ventricle and with it very closely connected heart failure, myocardial infarction, and cerebrovascular accidents (hereafter CVA) whether of haemorrhagic or ischaemic aetiology, changed renal function, and adverse effects on course of gravidity. Degree of risk of the high blood pressure is reflected by its first position in the Table of 7 the Most Important Risk Factors published by WHO, which are determining a prognosis of cardiovascular diseases. Degree of risk grows both as a result of growth of values of the blood pressure and growth in number of risk factors.

Concerning blood pressure (hereafter BP) values, value of systolic blood pressure (hereafter SBP) is considered to be of higher risk then value of a diastolic BP. Increased activity of the sympathetic nervous system and renin-angiotensin system leads to increased pulse rate, increased cardiac output, and increased peripheral vascular resistance and thereby SBP values. There is a proved relationship between SBP values and important cardiovascular diseases such as CVA, heart failure, renal failure, hypertrophy of the left ventricle, and ischaemic heart disease.

MRFIT study, published by Stamler in 1993, demonstrated in almost 350 000 cases that growing SBP values more significantly increase risk of death from CVA than growing values of diastolic BP.

Likewise hypertrophy of the left ventricle, emerging particularly along with uncontrolled hypertension and development of which is affected particularly by SBP values, increased pulse rate, and peripheral venous resistance, is also an important risk factor of heart failure, myocardial infarction, and sudden death. Thus we can say that the ill with hypertrophy of the left ventricle are on a higher risk and thereby have poorer prognosis than those with its regression as a result of treatment. Degree of risk of SBP value confirmed results of The Cardiovascular Health Study published in 2001. They imply that SBP is better predictor of cardiovascular accidents than diastolic BP or pressure systolic-diastolic amplitude.

Important role of high blood pressure in aetiology of cardiovascular diseases is thus undeniable. The most important means of prevention to this condition is its efficient control.

Prognosis of hypertension is proved to be more dependent on the level of blood pressure during treatment than before its initiation. There exist a lot of evidence that effective treatment of hypertension brings much better prognosis for the ill. Based on meta-analyses of clinical studies WHO evaluated decline of risk rate and decline of acute cardiovascular accidents in relation to lowered BP values by 10/5 mmHg and 20/10 mmHg in 1000 patients over a 1 year period. The biggest gain from lowered BP had patients with a very high risk of cardiovascular diseases with pressure values lowered by 20/10mmHg.

Some other studies have also brought compelling results to this issue.

HOT study has revealed, besides pinpointing optimal BP values - 148,5 mmHg in systolic and 82,6 mmHg in diastolic component - decrease in risk of significant cardiovascular accidents by 25% and 30% when end values of systolic and diastolic pressures were achieved.

Also the PROGRESS study, with its conclusions published in 2001, revealed that secondary prevention of recurrent CVAs´ geneses is directly related to lowered blood pressure. This study has also found out that lowering BP by means of treatment is important for primary prevention of serious cardiovascular diseases, including acute myocardial infarction (risk is 26% lower). Clinical experiences confirm conclusions from above mentioned studies that risk of systolic BP in relation to CVA and IM in individuals with treated arterial hypertension is lower than in individuals who are not treated.

Evolution of epidemiological situation in the Czech Republic and in previous Czechoslovak Federation is documented by decrease in mortality resulting from cardiovascular diseases. During the last 15 years there has been decrease in mortality by more than 20% and developmental tendency is permanently of a decreasing nature. We were interested in situation of the two the most important and the most frequent cardiovascular diseases - myocardial infarction (hereafter MI) and CVA.

MI mortality (figured out for 100 000 citizens) had made a strong fall by 1997. However, there was a slight increase since 1998 which in 2000 stabilised at the level of 1998. Nevertheless, there has been decrease in mortality from this circulatory disease by 34,4% over the last 15 years.

Very similar, although finally different, is progress of mortality from CVA which in contrast to MI has been gradually though slightly growing since 1997. So that almost 40% decrease in 1997 was in 2000 only 31%. In more detailed investigation of this phenomenon we have learned that among people who died from CVA the majority were women, namely in all the years following 1997 and in groups of people over the age of 70. Before the age of 70 there are slightly more men among these people.

There are certainly many possible causes accounting for this situation. We can name bigger prevalence of arterial hypertension in women over the age of 55 when in 70 years old and older women the prevalence is, compared to men, higher by 13 - 15%. Average values of SBP also grow parallelly to the age while diastolic BP values in 60 years old and older people show permanent fall. Increase in average

age of women compared to men has been also very important factor during the last years with regard to statistical data. There are also some other causes which may affect present state, such as worsened life style, including eating habits and the last but not least worsened care of people with hypertonia where reduced adherence to treatment is for this age group typical.

In the end we can summarise that the most important task which can have positive impact on mortality from CVA and other cardiovascular diseases is to provide more care to people with high blood pressure. This task implies not only to improve seeking of people with high BP but also to improve levels of diagnosing and treatment of our patients consistently with recommendations of the Czech Hypertension Society. That is the only way how to stop adverse evolution of mortality from the two, over the last years, the most important diseases of circulatory system.

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CHRONOTHERAPY EFFECTIVENESS IN TREATMENT OF MALIGNANT DISEASES

Rostislav Vyzula

Comprihensive Cancer Care Department, Faculty of Medicine, Masaryk University, Masaryk Memorial Cancer Institute

Address correspondence to:

Doc.MUDr.Rostislav.Vyzula,CSc.
Head,
Comprihensive Cancer Care Department,
Faculty of Medicine, Masaryk University
Masaryk Memorial Cancer Institute
Žlutý Kopec 7
656 53 Brno

Cancer takes time, usually the better part of an average human life span, to develop and usually many years to kill. This simple observation indicates that earlier cancer detection is possible. It indicates that there is a substantial span during which a wide range of complementary treatments may be used to cure a cancer or to control it if it cannot be cured.

The living unit of the body gives rise to cancer is the cell. The cell chooses the cancer path against heavy odds. Many injuries to the cell's reproductive machinery, its DNA, are required in order to give rise to the family of cells eventually recognized as a cancer. All of these injuries must be endured by the cell without killing it or making it unable to reproduce and none of them can be repaired. The cell that becomes injured, however, has genetic programs that command it to commit suicide if its genetic material is irreparable damaged, as well as complex genetic programs that command it to fix almost any repairable injury to its DNA. If any of the injuries required for cancer can be prevented or repaired, or if cells with these injuries can be selectively killed or cajoled into committing suicide, the development of that cancer will be thwarted.

There are natural cycles that are of major importance to the delicate balance between a person and an incipient or established cancer. These cycles include seasonal cycles, fertility cycles, and daily or circadian cycles. This rhythmic biologic time structure has evolved to maximize the stability of living things and to ensure that they do not waste precious energy. Life´s absolute need for stability requires continuous readjustment of the system to external and internal dynamic requirements. This may be pictured as cyclical activation/production, stability assessment, triangulation, and reinitiation cycle. The cyclical nature of the coordination in time provides that if an essential task is not completed, in a timely fashion, the organism has a second chance to do so within a defined span

(one cycle length). Escape from this sort of temporal ordering has lethal consequences.

Casual observation of biologic time structure reveals certain fundamental frequencies more or less tightly tied to external and internal environmental regularities. The internal genetically determined, circadian and menstrual cycles are a fair example of two primary rhythms tightly tied to internal genetic time-keeping mechanisms essential for organismic and special survival, respectively. The yearly cycles, which are made clear by the seasonal changes in both our biology and our environment, are also essential temporal reference frames and interact with all other biologic frequency ranges.

The toxicities of more than a score of the most commonly used chemotherapeutics agents have been shown to be circadian stage dependent in murine systems. The anticancer efficacy of many of these agents, either given singly or in combination, has also been shown to be circadian time-dependent in experimental studies. This reproducible temporal variability in antitumor effect and normal tissue toxicity can be explained in part by two important observations. First, experimental and clinical studies have documented that the pharmacokinetics of many anticancer drugs, show consistent and reproducible circadian temporal variation depending on the time of their administration. Second, most normal tissues are reproducibly and rhytthmically more and less senstitive to the effects of drugs at specific times of day. Finally, some tumors exhibit similar rhythmic susceptibility patterns, while others seem to vary little during the circadian cycle. These circadian rhythmic variations in drug pharmacokinetics and tissue susceptibility may be exploited to select a time for treatment that results in an increased tumor-cell kill and reduced toxicity.

Chronomodulated infusion of fluorouracil (5-FU), leucovorin (LV) and oxaliplatin (L-OHP) has repeatedly achieved objective response rates greater than 50% in phase II and III trials in metastatic colorectal cancer. This figure exceed by more than 20% those achieved with 5-FU, 5-FU-FA or irrinotecan in multicentric trials. The concept of chronomodulated delivery of chemotherapy is based on circadian rhythms. These rhythms in target tissues may account for the fact that increased tolerability results from 5-FU dosing in the first half of the rest span and cisplatin (CDDP) in the late activity span. Median survival in metastatic colorectal disease with standard therapy is between 12-15 months. Encouraging data with chronomodulated infusion of fluorouracil, leucovorin and oxaliplatin in the literature led us to perform the study with CDDP, due to lack of oxaliplatin in the Czech Republic. There were 80 patients included in the study, and 53 of them were evaluable (25 men, 28 women) with gastrointestinal cancer (colorectal carcinoma 41, gastric and pancreatic carcinoma 4, esophageal carcinoma 3, gallbladder carcinoma 1). We administered combination of 5-FU/CDDP/LV in chronomodulated schedule. 5-FU was gradually administered in 12 hour infusion from 22:00 to 10:00 in two to three time periods with the maximum dose around 04:00 AM, and CDDP was gradually administered in 12 hour infusion from 10:00 to 22:00 in two to three time periods with the maximum dose around 04:00 PM. Our current results are as follows: PR 20,8%, SD 41,5%, PD 35,8%, and follow up from 2,5 to 18 months. The regime was very well tolerated with leukopenia gr III in 0,4%, trombocytopenia gr III in 0,4%, neurotoxicity gr II in 15,1%, and gastrointestinal

toxicity in 5,7%. In conclusion our small study confirmed very well tolerable regime with good response rate in very advanced metastatic gastrointestinal malignant disease.

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HEART RATE VARIABILITY IN PATIENTS WITH CHRONIC ISCHEMIC HEART DISEASE: EFFECT OF 8-WEEK EXERCISE TRAINING

Jančík Jiří, Várnayová Leona, Siegelová Jarmila, Svačinová Hana, Dobšák Petr, Placheta Zdeněk, Toman Jiří*, Meluzín Jaroslav*, Panovský Roman*

Dept. of Functional Diagnostics and Rehabilitation and *Ist Dept. of Cardioangiology, Faculty of Medicine, Masaryk University, St.Anna Teaching Hospital, Brno, Czech Republic

Address correspondence to:

MUDr. Jiří Jančík
Department of Functional Diagnostic and Rehabilitation
Faculty of Medicine, Masaryk University
Pekařská 53
656 91 Brno

INTRODUCTION

Low heart rate variability is an indication of increased risk of sudden cardiacdeath after infarctus myocardium even in patients with chronic heart failure (1,2,3,4). On the basis of this knowledge this risk factor is to be modified by physical training. Whereas the results of these works are similar if variability is expressed in time domain, i.e. as the magnitude of standard deviation of pulse interval duration, in frequency domain the results are controversial (5).
The study was aimed at finding out whether the specific training programme at the level of anaerobic threshold can influence the heart rate variability expressed in frequency domain in patients with chronic coronary arteries disease (CCD).

PATIENTS AND METHODS

We have examined fifteen patients with CCD altogether (coronarography, NYHA I-II, age±SD: 61±7 years, SPECT, echocardiographical examination on the apparatus SONOS 5500, Hewlett Packard, USA, ejection fraction 50±7%) before and after 8 weeks´ training. The treatment regime of the patients was optimized according to the symptoms of the disease. The standard medical treatment between the first examination and the second one consisted of ACE inhibitors, diuretics, nitrates and beta-blockers in different combinations.
Criteria of including the patients into the study
15 patients with chronic ischemic heart disease complying with the following criteria were included into the study:

1. coronarographically verified significant stenosis of at least one coronary artery - stenosis of more than 50% of the diameter of lumina
2. presence of ischemia was proved by the physical training test (positive SPECT and ergometry) and verified coronarographically. The patients were then indicated for the conservative therapy

3. the patients were not submitted to CABG in the last six months
4. they did not suffered from infarctus myocardium in the last three months
5. PTCA was not carried out in the last three months
6. haemodynamically significant heart defect is not present
7. manifest heart failure is not present
8. unstable AP is not present
9. written informed approval

Criteria of exclusion from the study
1. manifest heart failure
2. unstable angina pectoris
3. condition after PTCA in the last three months
4. condition after the surgery revascularization intervention in the last six months
5. haemodynamically significant heart defect
6. diabetes mellitus treated by insulin
7. disease preventing from the completion of the rehabilitation programme

Spiroergometry up to the maximum limited by the symptoms was carried out before the training and after it. We evaluated the maximum performance (Wmax) and cardio-respiratory indices (intake of oxygen VO2 max, MET, heart rate). The first spiroergometry was applied for the determination of anaerobic threshold (ANP) to decide on the training intensity. The group performed the physical training at the level of anaerobic threshold.
The training was realized at the Department of functional diagnostics and rehabilitation three times a week and consisted of 15 minutes of the warming period with individual exercises followed by 20 minutes of cycling on ergometer with a lower and higher intensity, then 15 minutes of cooling down took place. The rehabilitation programme was performed by the patients for eight weeks.
The heart rate variability was examined by the apparatus Varia Puls T3. We used a short-time evaluation of the heart rate variability in the supine and standing position at spontaneous breathing and in the supine position at breathing according to the metronome with the frequency 0,33 Hz. The obtained results were evaluated by spectral analysis. An example of examination can be seen in Fig. 1. The examination was performed before the beginning of the training (A) and after 8 weeks of the training (B).
Both parametrical and non-parametrical tests were used for statistical evaluation of partial results. The total evaluation was made according to the statistical method ANOVA.

RESULTS

The results are given as average values ±SD. Table 1 gives rest values of the heart interval duration (SI ms), maximum reached performance at the cycling ergometry (W_{max}), intake of oxygen (VO_{2max} ml.min-1).
The heart rate variability is evaluated as the total performance (TP, ms^2), performance at low frequencies (LF, 0,04-0,14 Hz, ms^2), performance at high frequencies (HF, 0,15-0,4 Hz, ms^2) and ratio LF/HF (Table 2).

Table 1. Rest values of the heart interval duration, maximum reached performance at the cycling ergometry and maximum intake of oxygen

	SI	W_{max}	VO_{2max}
A	953±130	142±31	1486± 343
B	1009 ± 93*	156±32*	1612±360*

(* $p<0.05$)

Table 2. Spectral analysis of heart rate variability

	TP	LF	HF	LF/HF
A	299 ±207	113 ± 94	194 ± 177	0,85 ± 0,92
B	838± 1612*	455 ±1224	390± 383*	1,04 ± 1,01

(* $p<0.05$)

Our results after the 8 weeks´ rehabilitation programme show significant differences in extension of heart intervals at rest. The cycling ergometry after the 8 weeks of training showed increased maximum performance and intake of oxygen in comparison with the examination before the training, the differences being statistically significant at the 5 % level of significance. Spectral analysis showed increased total performance of heart rate variability after the 8 weeks of training in comparison with the analysis before the training and increased performance at high frequency, both at the 5 % level of significance.

DISCUSSION

The increase of heart rate variability is usually connected with rising of parasymphathetic activity. It is therefore not surprising that the increase was observed in our work in the same way as in the works of other authors. It is surprising that the increase of variability at high frequency was not observed by some authors (6,7) even if variability at high frequency corresponding to respiration arrhythmia is mediated only by vagus nerve. We did observe the increase of variability at high frequency. Unlike other authors who evaluated variability on the basis of 24 hours´ EKG record we evaluated in our work short-time variability but at the controlled breathing frequency. With regard to the fact that the amplitude of respiration arrhythmia depends in a great measure on the frequency and depth of breathing, where the breathing frequency is not controlled variability at high frequency can be submitted to non-predictable changes.

Decreased heart rate variability has been considered in the last twenty years to be a significant risk of death of the patients after infarctus myocardium (1,2,3). In prediction of total deaths of the patients after infarctus myocardium decreased heart rate variability is comparable to the significance of the magnitude of ejection fraction in the risk stratification (4).

CONCLUSIONS

Up to the present the matter of anaerobic training influence on the performance of patients and autonomous nervous system has not been fully explained. Our results have shown that the 8 weeks´ training at the anaerobic threshold level leads to the increase of functional capacity of the patients with the chronic disease of coronary vessels. The increase of the total spectral performance and the increase of the performance in the range of high frequencies are symptoms of the increase of parasympathetic tonus after eight weeks of the controlled training. The found out extension of the heart interval in time domain is also symptomatic for the increase of parasympathetic tonus.

Our work completes the knowledge of the significance of rehabilitation training in the patients with the chronic disease of coronary vessels.

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Fig.1

Example of graphical representation of the results of spectral analysis of heart rate variability in 50 years old woman with chronic ischemic heart disease by the system VariaCardio TF3 (A - before rehabilitation, B - after the completion of hospital training).

Axis x - frequency of oscillations of intervals RR (Hz).

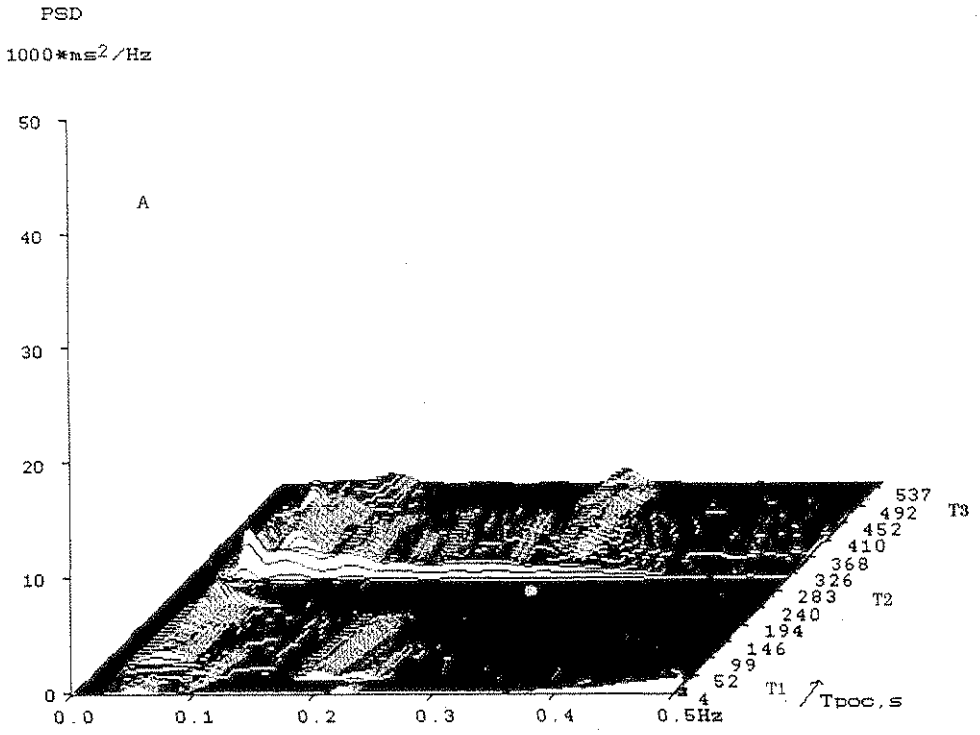
Axis y - power spectral density ($100ms^2$. Hz^{-1}).

Axis z - time (s).

T1,T2,T3 - sections of RR registered in supine, standing and supine positions at controlled breathing.

A - variability is apparently reduced in all frequency bands.

B - after the completion of the hospital controlled rehabilitation programme increase of VSR mainly in high-frequency band of spectrum (corresponds to the increase of VSR modulation by parasympathetic nerve).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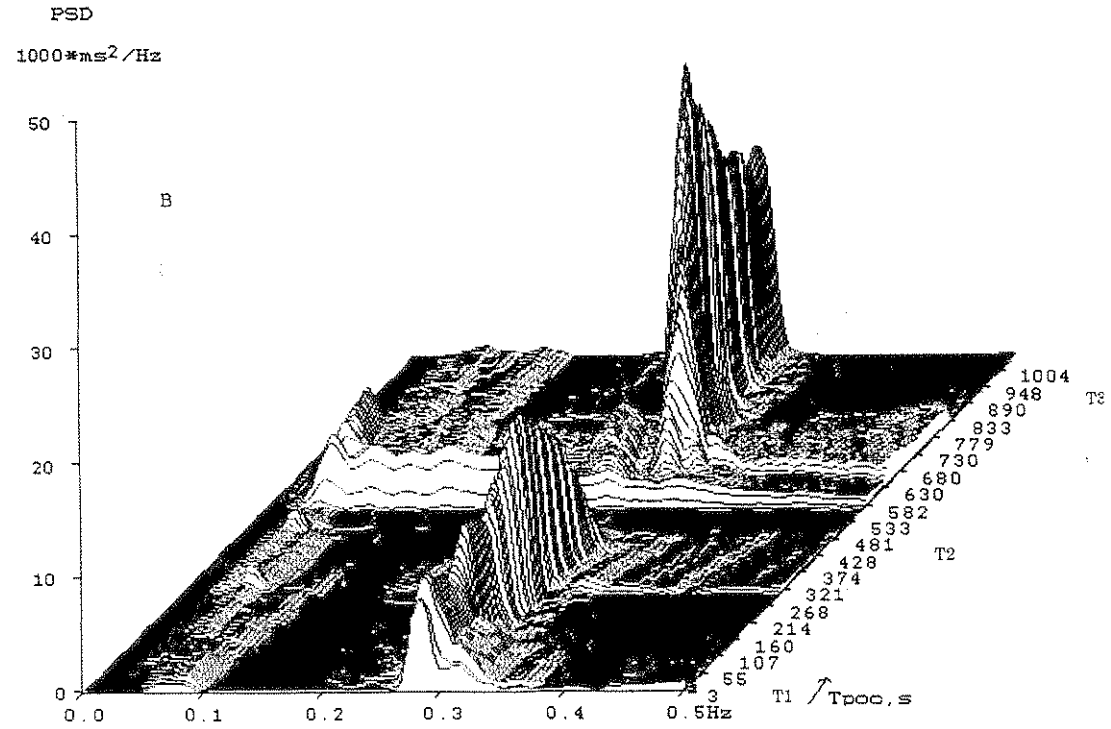
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CHANGES IN AEROBIC EXERCISE CAPACITY IN PATIENTS WITH CHRONIC ISCHEMIC HEART DISEASE: EFFECT OF 8-WEEK EXERCISE TRAINING

Jančík Jiří, Dobšák Petr, Kožantová Lucie, Siegelová Jarmila, Svačinová Hana, Vank Pavel, Placheta Zdeněk, Toman Jiří*, Meluzín Jaroslav*, Panovský Roman *

Dept. of Functional Diagnostics and Rehabilitation and *Ist Dept. of Cardioangiology, Faculty of Medicine, Masaryk University, St. Anna Teaching Hospital, Brno, Czech Republic

Address correspondence to:

MUDr. Jiří Jančík
Department of Functional Diagnostic and Rehabilitation
Faculty of Medicine, Masaryk University
Pekařská 53
656 91 Brno

INTRODUCTION

Regular aerobic exercise training can initiate a number of positive adaptation changes in patients with chronic ischemic heart disease (1,2) and reduce morbidity and mortality of the patients (3,4,5). According to a majority of authors only an intensive aerobic load can initiate these changes. Only few papers dealing with exercise training with a low load intensity have been published. If the exercise training with a low intensity could initiate changes being comparable with those initiated by exercise training with a high intensity, the possibility of rehabilitation at low levels of the load would remove fear both of doctors and of patients of possible negative consequences of high intensity of exercises and the exercise treatment could be more accessible and more simple.

The work was aimed at evaluation of influence of 8-week exercise training of different load intensity on aerobic exercise capacity of the patients with chronic ischemic heart disease and reversible ischemia of myocardium at load.

PATIENTS AND METHODS

32 patients (NYHA I-II, age-average ±SD: 63±8 years) with stable chronic ischemic heart disease were included in the study. Reversible ischemia was established in all patients by positive perfusion scintigraphy of myocardium (SPECT). The ischemic disease was verified coronarographically by proving of at least one haemodynamically significant coronary stenosis (stenosis of more than 50 % diameter lumina). All the patients were intended for conservative therapy and it was not changed in the course of intervention. The protocol of the study was approved by local ethical commission and the patients signed informed approval. The patients had a stable form of the disease. We consider the disease to be stable

when it was without manifestations of haemodynamical, ischemic and electrical instability, in the patients 6 months after cardiac surgery, at least 3 months after PTCA or acute myocardial infarctus, without haemodynamically significant valve defect. We did not include the patients with diseases contra-indicating exercise treatment.

Aerobic exercise capacity was examined in morning hours always at the same time before the patients were included into the study and after 8 weeks of aerobic training. The symptom- limited spiroergometry started by 3-minute adaptation sitting on bicycle ergometer followed by 2-minute loads from 20 W being increased in 2-minute intervals always by 20 W, in the course of which ventilation and respiration parameters were measured; they were evaluated by the instrument Pulmonary Function System 1070 (MedGraphics,USA). We determined the value of the anaerobic threshold (ANP) serving for prescribing a suitable load intensity that we expressed, for the purposes of rehabilitation, in watts, in the corresponding value of the heart rate, and by degree of Borg scale of subjective perception of the load intensity. During the whole examination we monitored a 12-lead electrocardiogram (Schiller CS 100). Generally recognized criteria, in addition to reaching the symptom-limited maximum, were used for finishing the test. The loading capacity was expressed in values of the maximum reached power in watts (Wmax), maximum peak oxygen uptake (VO2max) and multiples of oxygen uptake at rest - in metabolic equivalents (MET).

Before starting the rehabilitation the patients were randomized into 2 groups. Group A did exercises at ANP level (training of high intensity), group B at the level of 60 % ANP (training of low intensity). The ANP level was expressed in watts.

The training was carried out in the Department of Functional Diagnostics and Rehabilitation of St. Anna Teaching Hospital in Brno three times a week and consisted of 15-minute heating phase followed by 20-minute aerobic exercise on ergometer, then 15-minute cooling phase followed. The rehabilitation programme was performed by both groups for the time of eight weeks.

Statistical evaluation of partial results was carried out by both parametrical and non-parametrical tests. The total evaluation was made according to the statistical method ANOVA, Wilcoxon.

RESULTS

The results of spiroergometry (average ± SD) in randomized groups A and B of the patients with chronic ischemic heart disease with reversible ischemia of myocardium at load are given in Tables 1 before and 2 after 8 weeks of exercise training.

Table 1.

A. Training with load intensity at the anaerobic threshold level (EF = 51.5±6%, n=14)

	W_{max} (W)	VO_{2max} (ml O_2STPD	MET_{max}
1	144±32	1886±321	6,3±1,6
2	160±38*	2001±359*	6,8±1,4*

(* p < 0.05, 1 before the training, 2 after the training)

Table 2.

B. Training with load intensity at 60 % of the anaerobic threshold (EF = 48±13%, n=18)

	W_{max} (W)	VO_{2max} (ml O_2STPD)	MET_{max}
1	110±34	1461±316	4,9±0,8
2	118±36*	1585±377*	5,3±1

(* p < 0.05, 1 before the training, 2 after the training)

8-week training of the patients with stable chronic ischemic heart disease with both high and low intensity increased the maximum reached power and improved the transport system capacity expressed by the value of the maximum oxygen uptake.

DISCUSSION

Physical training has been used at present routinely in rehabilitation of cardiac patients as an important part of the treatment. It leads to improvement of physical fitness (6,7), psychosocial functions (8), to the decrease of sympathetic activity and to positive adaptation changes of metabolism and skeletal musculature (9). A majority of current papers proceed from the assumption that only a high intensity load is sufficient for initiating these changes. Only few studies compare the influence of training with a different load intensity. Belardinelli et al.(10) described increase of maximum oxygen uptake and anaerobic threshold level after 8 weeks of training with the load intensity corresponding to 40 % level of maximum oxygen uptake in the patients with stable chronic heart failure. According to Blumenthal et al. (11) the training with low load intensity in the patients after myocardial infarction gave rise to similar changes of maximum oxygen uptake like the high intensity training. Worcester et al.(12) described a favourable effect of the training with a different load intensity on the quality of life after acute myocardial infarction. Our study evaluates the influence of 8-week aerobic training with high and low load intensity in the patients with stable forms of chronic ischemic heart disease. In accordance with the results of papers of the above-mentioned authors we found out in both randomized sub-groups statistically significant increase of maximum reached power and improvement of function of the transport system.

CONCLUSIONS

After the 8-week training with a load intensity both at the ANP level (group A) and at the level of 60% ANP (group B) maximum reached power and maximum oxygen uptake were increased in the patients with chronic ischemic heart disease We can assume, therefore, that even a low load intensity could be effective in rehabilitation of these patients. The matter of utilization of low load intensities in rehabilitation of cardiac patients will require, however, further research and comparison of larger homogenous sets.

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LOW-FREQUENCY ELECTRICAL STIMULATION OF THE STRENGTH MUSCLES COULD IMPROVE THE IMPAIRED METABOLISM AND BLOOD SUPPLY IN PATIENTS WITH CHRONIC HEART FAILURE

Dobšák Petr, Eicher Jean Christophe*, Jančík Jiří, Siegelová Jarmila, Kožantová Leona, Várnayová Lucie, Svačinová Hana, Vohlídalová Ilona, Sosiková Michaela

Department of Functional Diagnostics and Rehabilitation, Faculty of Medicine, Masaryk University Brno, St. Anna Faculty Hospital Brno, Czech Republic
*Dept. of Cardiology II, University of Burgundy, Dijon, France

Address correspondence to:

MUDr.Petr Dobšák,CSc.
Department of Functional Diagnostic and Rehabilitation
Faculty of Medicine, Masaryk University
Pekařská 53
656 91 Brno

INTRODUCTION

The chronic heart failure (CHF) is often accompanied by global hypoperfusion which affects a great part of the skeletal muscle mass. The intensity of catabolism increases, the reactive oxygen species and a large scale of circulating cytokines stimulate the apoptosis development. The skeletal muscle oxidative metabolism is depressed, following to low intracellular pH levels, increased phosphocreatine depletion during exercise and decreased phosphocreatine resynthesis [1]. Increased sympathetic tone and stimulation of the renin-angiotensin-aldosterone system influence the redistribution of regional blood flow and create the endothelial dysfunction both in large and resistance vessels. This leads to an impaired peripheral vascular dilatation in response to vasodilator stimuli and reduction of blood flow and O2 supply in skeletal muscles [2]. The chronic hypoxia damages strongly the structural and metabolic integrity of the muscle fibers. The resulting general atrophy decreases the muscle power and fatigue resistance. Sometimes this situation progress to so-called cardiac cachexia. The benefitial influence of the exercise training on the aerometabolic capacity and fatigue tolerance in patients with CHF has been repeatedly reported. However the commonly used trainings are based on systemic exercise and could not be tolerated by all patients with CHF, es pecially by those with a severe grade of failure or with life-threatening arrhythmias. A new approach in the field of cardiac rehabilitation is represented by the method of low-frequency electrical myostimulation (LFMES) of the skeletal muscles. In in-vitro conditions the LFMES of 10Hz changes the phenotype of the stimulated mammalian skeletal muscle fibers. LFMES transforms the myosin chains of the "fast" type to the type "slow", characterized by a higher resistance to fatigue [3, 4]. LFMES also increases the capillary density and enhances the

perfusion in rat and rabbit strength muscles [5, 6]. The most important is the fact that all these experimental results could be applicable also in humans conditions. The aim of our study was to evaluate the long-term effect of LFMES on the metabolic performance and on the supposed perfusion changes in skeletal muscles in patients with chronic heart failure.

PATIENTS AND METHODS

Patients

A group of 12 patients (class NYHA II-IV, men and women, after coronarography, mean age 56 ± 9years, EF = 22 ± 9%), with optimized pharmacologic treatment (ACEI, betablockers, diuretics) and symptomatically stable, was evaluated.

Protocole of LFMES application

The stimulated muscles were both quadriceps and calf muscles. Special rectangular electrodes (80x100mm) were positioned on the thigs and the calves. The electrical stimulation was performed 1 hour/day, 5 days a week for 5 weeks, using dual-channel stimulators Elpha 2000 (Danmeter, Odense, Denmark). The stimulators delivered a biphasic current of 10Hz frequency. The pulse duration was 200msec with "on-off" mode of stimulus (20sec stimulation, 20s pause). The maximal stimulation amplitude was 60mA.

^{31}P MRS study

To evaluate the muscle metabolic patterns, a phosphorus-31 nuclear magnetic resonance spectroscopy of gastrocnemius muscle (^{31}P MRS) was performed. The exercise protocole for ^{31}P MRS consisted in repeated plantar flexions of equal amplitude at a frequency of 0.5Hz against a calibrated load. The initial workload generated 1W and was increased by 0.25W every 3 minutes. Spectra were collected every minute; the protocole was ended at fatigue. The following recovery period was monitored during 10min period and a spectrum was enregistered every 30 seconds. The relative concentration of inorganic phosphates (Pi) and phosphocreatine (PCr) were calculated by integration and corrected for different saturation. Changes in PCr concentrations were expressed by the ratio PCr/PCr+Pi. The PCr recovery rate was calculated and expressed using the time constant (1/k) by a standard protocole described by *Hanada et al.* [7].

MRI study

The volumes of soleus and gastrocnemius muscles were determined by the method of magnetic resonance imaging (MRI) using a FLASH 2D (gradient echo) sequence in the axial plane with a pulse repetition time = 600msec, echo time = 10msec, slice = 10mm with 5mm interslice gap and total number of slices = 26. Finally, a planimetric estimation of mucle cross-sectional area was performed and the total muscle volume was calculated.

All nuclear magnetic resonance measurements (^{31}P MRS and MRI) were obtained using a Siemens 1.5 T Magneton imaging system (Erlangen, Germany).

Bood flow velocimetry measurement

To evaluate the changes in peripheral perfusion, a standard pulsed-wave Doppler [8] of right femoral artery was performed before and after the end of the 5 week period of stimulation using a Sonos 2000 echograph (Hewlett Packard, Andover,

USA). The measurements were performed before each session after 15min of rest, and after 15min of stimulation (during the "off" 20sec period). Mean value of blood flow velocity (cm/s) was calculated from 5 measurements.

Statistics

A Wilcoxon paired test was used for statistical analysis. The statistical significance was defined as a $P < 0.05$.

RESULTS

The analysis of the muscle metabolic parameters using the ^{31}P magnetic resonance spectroscopy of gastrocnemius muscles did not show some essential changes. There was only a slight tendency to diminution of the time constant (1/k) of PCr post-exercise resynthesis after 5 weeks of LFMES, but without statistical significance **(Table 1.)**.

Table 1. Results of 31P MRS analysis of gastrocnemius muscle before and after 5 weeks of LFMES (all values are expressesd as mean ± SD).

Muscle:	Before 5 weeks of LFMES	After 5 weeks of LFMES	*P* value (Wilcoxon paired)
PCr/PCr+Pi at rest:	0.88 ± 0.27	0.89 ± 0.35	NS
Maximal workload (W):	1.69 ± 0.53	1.92 ± 0.1	NS
PCr/PCr+Pi at maximal workload:	0.51 ± 0.1	0.52 ± 0.1	NS
PCr resynthesis time constant (1/k):	81.1 ± 79.1	53.2 ± 28.2	NS

(NS – non significant)

In contrast to these results, the analysis of muscle volumes by MRI proved a significant increase of the muscle mass in both soleus and gastrocnemius muscles after 5 weeks of LFMES in comparison with values before rehabilitation (**Table 2.**).

Table 2 Results of MRI analysis of muscle mass volumes of soleus and gastrocnemius muscles (cm3) before and after 5 weeks of LFMES.

Stimulated muscle:	Before 5 weeks of LFMES	After 5 weeks of LFMES
soleus muscle	315.2 ± 65	331.5 ± 44
gastrocnemius muscle	254.3 ± 47	278.6 ± 38 *

(- P < 0.05)*

The Doppler velocimetry measurements showed a significant increase of blood flow velocity in right femoral artery in 15th minute of stimulation after 5 weeks of

LFMES in comparison with the flow velocity before the rehabilitation with LFMES (**Table 3.**, **Fig 1.** and **Graph 1.**). The differences in the blood flow at the rest period before and after 5 weeks of stimulation did not differ statistically.

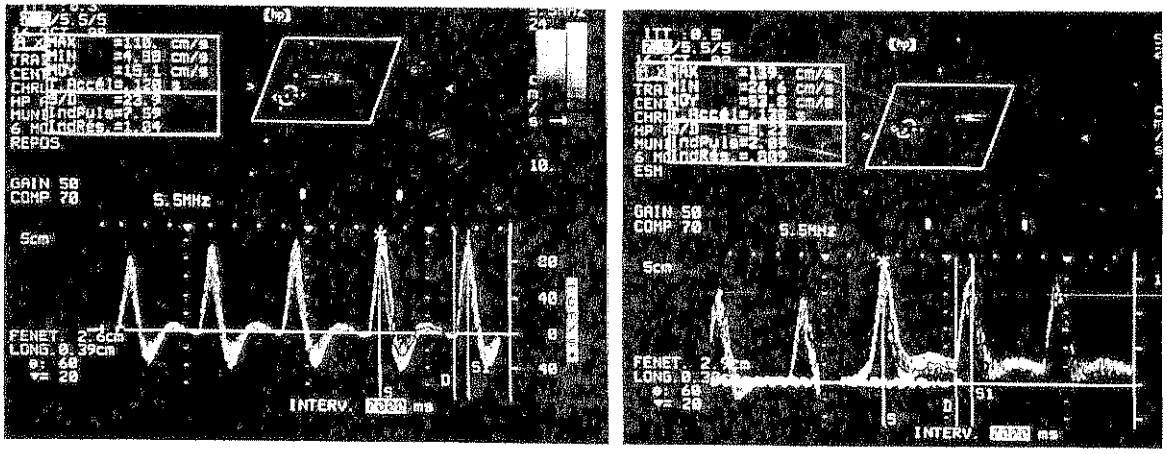
Table 3. Individual results of Doppler velocimetry of blood flow in right femoral artery (at rest and in the 15th minute of stimulation) before and after 5 weeks of LFMES.

Patients group (n = 12)	Before 5 weeks of LFMES		After 5 weeks of LFMES	
	Flow velocimetry at rest (cm/s)	Flow velocimetry in 15th min of LFMES (cm/s)	Flow velocimetry at rest (cm/s)	Flow velocimetry in 15th min of LFMES (cm/s)
1.	6.4	8.5	8.3	7.6
2.	6.1	14.4	6.2	24.1
3.	15.6	68.8	16.1	63.7
4.	16.8	37.2	10.7	43.1
5.	5.8	28.2	8.6	51.6
6.	13.7	12.7	14.5	53.2
7.	10.2	71.2	16.9	104.0
8.	8.6	22.1	9.0	27.5
9.	16.3	51.2	19.8	97.5
10.	9.9	33.3	8.4	45.4
11.	25.6	98.7	18.4	80.0
12.	7.9	45.9	17.2	60.0
Mean:	**11.92833**	**41.02167**	**12.81917**	**54.07167 ***
(± SD):	***5.900252***	***27.47455***	***4.745822***	***28.97922***

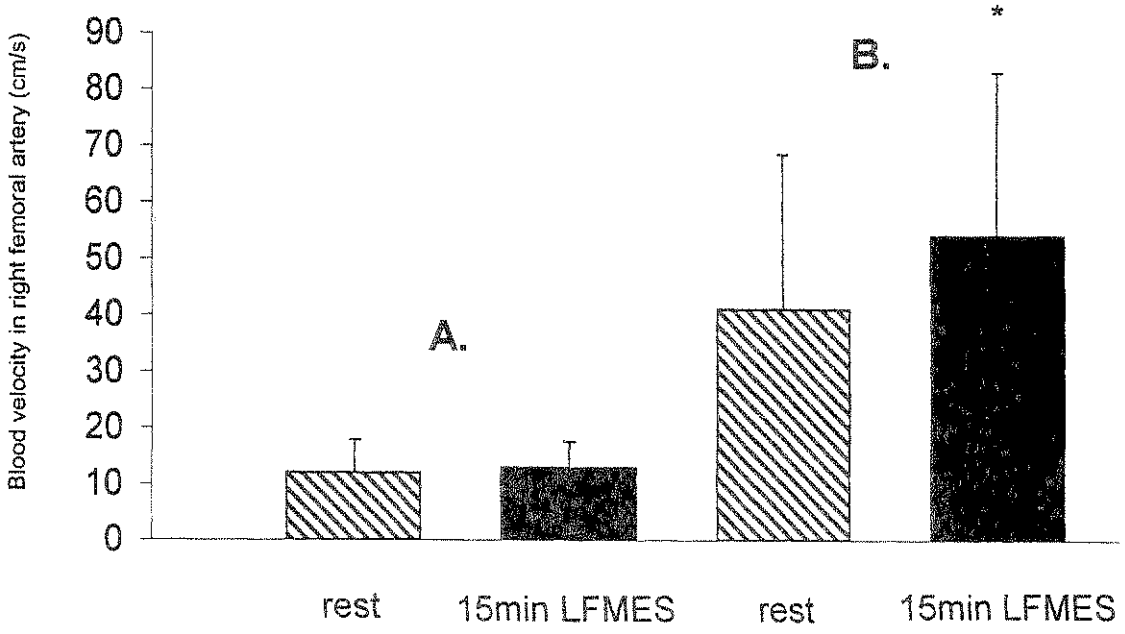
(= P < 0. 05)*

Fig. 1 Example of Doppler blood velocimetry measurement in patient No.6 after 5 weeks of LFMES. A. - mean blood velocity at rest (15.1 cm/s), B. - mean blood velocity after 15min of stimulation (53.8 cm/s).

A. B.



Graph 1. Comparison of the mean values (± SD) of blood flow velocity (cm/s) in right femoral artery before (A.) and after (B.) 5 weeks of LFMES (- $P < 0.05$).*



DISCUSSION

The use of chronic low-frequency stimulation in experimental models helped to study the muscle plasticity. It seems that LFMES involves qualitative and quantitative changes in different elements of the strength muscle fibers. Both structural and functional alterations could be probably caused by transformations of fast protein isoforms to their slow counterparts, followed by an increased activity of oxidative enzymes, improved oxygen consumption and growth of the terminal microvascular bed. These changes represent the basis for the general improvement of muscle metabolic patterns, including the diminution and prevention of atrophy and increased resistance to fatigue. Such an events could play a very important role in the situation of chronic heart failure which is typically characterized by impaired oxidative capacity.

The above presented data indicate that long-term low-frequency electrical stimulation of the skeletal muscles could improve some metabolic and circulatory parameters in patients with CHF. The previously published results showing a significant improvement of exercise capacity parameters in patients with CHF after 5 week lasting LFMES, including the VO_{2peak}, VO_{2AT} and 6min walking test **[9]**. Also a very similar results were obtained in the same study by the nuclear magnetic resonance (^{31}P MRS and MRI). The failure to show a significant improvement in ^{31}P MRS parameters could be attributable probably to the too small sample number. Nevertheless the significantly increased muscle volume mass of both calf and quadriceps muscles in our study seems to contribute to the global structural and functional (and very probably also metabolic) improvement.

The increase of the muscle mass after chronic low-frequency stimulation could be accompanied by the capillary growth **[10]**. The benefitial effects of LFMES on vascular functions are probably related to the effect of increased flow on the endothelium. An acutely increased blood volume increases flow-mediated stress on the vessel wall, which in turn, liberates NO from the endothelial cells. It seems that the electrical stimulation (and resulting muscle contractions) cause the same vascular reactions as seen after classic physical exercise, f.e. exercise-induced hyperemia in resistance vessels **[11]**. Therefore the chronic structural vessel changes may probably be regarded as an extension of the sustained changes in endothelial function resulting from exercise and may also be dependent largely on NO-related mechanisms **[12]**. The significantly increased blood velocity in the femoral artery in this study reflects the importance of the achieved global vascular benefit for the peripheral muscle mass after 5 weeks of LFMES. Also these results confirms the previously reported positive effects of LFMES use in patients with CHF **[13, 14, 15].**

CONCLUSIONS

We assume that LFMES is safe and well tolerated method, without some dangerous side effects. In a relatively short time it can induce a significant improvement of the skeletal muscles conditions in chronic heart failure, including the increase of muscle mass and blood perfusion. In some patients with CHF the LFMES could represent a possible alternative to classical exercise training and an occassion to improve their health conditions.

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WALKING EXERCISE IN PATIENTS WITH TYPE 2 DIABETES AND PARAMETERS OF THE TRANSPORT SYSTEM CAPACITY

Svačinová Hana, Olšovský Jindřich[1], Žáčková Viera[1], Siegelová Jarmila., Placheta Zdeněk, Jančík Jiří, Dobšák Petr, Homolka Pavel and Svoboda Ladislav

Department of Functional Diagnostics and Rehabilitation, [1]IInd Department of Internal Medicine, Faculty of Medicine, Masaryk University Brno, St. Anna Faculty Hospital Brno, Czech Republic

Address correspondence to:

MUDr. Hana Svačinová
Department of Functional Diagnostic and Rehabilitation
Faculty of Medicine, Masaryk University
Pekařská 53
656 91 Brno

INTRODUCTION

The importance of regular physical exercise as a part of therapy of diabetes of both types is sufficiently proved by its favourable influence both on metabolic condition and on important parameters of cardiovascular system (1, 2). A number of studies have also shown that regular physical exercise can decrease the risk of origin of type 2 diabetes in patients with risk factors 3, 4). Diabetes mellitus of type 2 is accompanied by obesity in more than 80 % and developed insulin resistance (IR) already at the time when the disease is found out. Insulin resistance is considered, together with other pathologic conditions belonging to so called metabolic syndrom (abdominal obesity, hyperinsulinemia, arterial hypertension, dyslipidemia, prothrombotic condition), to be a key risk factor leading to the acceleration of atherogenesis. Breaking of insulin resistance and increasing of sensitivity to insulin appear to be the main therapeutic objective in patients with type 2 diabetes that should reduce the risk of cardiovascular complications. A number of studies show that these patients have a low level of physical fitness in comparison with healthy individuals of the corresponding age and that this low aerobic capacity is associated with a number of cardiovascular risk factors. Increase of aerobic performance and favourable influence on these risk factors led to decreasing of insulinemia and this favourable effect of physical exercise (in addition to other influences) is probably connected with a better sensitivity to insulin. It is proved that a short-time intensive load brings about decrease of insulin resistance. This decrease, however, continues for about 72 hours, then IR returns to the original condition provided that physical exercise is not repeated. That is why regularity of physical exercise is accentuated in patients with type 2 diabetes (5, 6, 7). A number of authors have been dealing with the question of which type of physical exercise and of what intensity, duration and frequency has such an efficiency in patients with type 2 diabetes that would favourably influence cardiovascular risk factors and at the same time would be safe, easily accessible and acceptable for the

patients from the psychical point of view. From this standpoint walking is most frequently recommended for the patients with type 2 diabetes as the training of a low to medium intensity. A number of the studies where walking was used as a kind of training, however, give different results as to the significant influence on the most important metabolic and cardiovascular parameters (6, 7, 8, 9, 10). In spite of that Levine et al. (11) give in their summary of results of the studies of rehabilitation of cardiac patients that the training of a low intensity is theoretically safer than that of a high intensity as to a possibility of sudden heart events in cardiac patients including, however, many patients with diabetes. Home exercises of the intensity of 60 - 70 % of maximum heart rate can bring about a substantial improvement of functional capacity with a high degree of safety and compliance of patients. At that also a relatively small improvement of fitness acquired by training of a low intensity (particularly walking) can contribute to a significant decrease of cardiovascular mortality.

Our study was aimed at the evaluation of influence of walking with intensity at the level of anaerobic threshold on selected parameters of the transport system in patients with diabetes of type 2.

PATIENTS AND METHODS

The patients´group consisted of 11 patients with type 2 diabetes, 6 men and 5 women, treated in Diabetes centre of II. Dept. of Internal Medicine of St. Anna Teaching Hospital in Brno. Average age was 56,6 ± 8 years, mean BMI 30,5 ± 2, average time of duration of diabetes was 6,9 ± 5 years. All patients were treated either by diet and/or by peroral antidiabetics. No patient manifested severe degree of specific diabetic complications or other diseases contraindicating the selected physical activity. 6 patients had controlled hypertension treated by Ca antagonists and/or ACE inhibitors. A long-term satisfying metabolic compensation was a condition of participation in the study; the patients with any treated form of ischemic heart disease were not included.

After the internal examination (anamnesis, physical examination, ECG at rest, basic laboratory examination) all patients were submitted to **initial** bicycle spiroergometry (Cardiovit CS - 10 Schiller, gas analyzer Medgraphics - Fig.1) to determine their functional fitness, to eliminate ECG signs of ischemic heart disease and to determine the limit of a safe intensity of exercises. We chose the protocol with intensified workload up to the symptom-limited maximum: basic load 40 W, intensification at 20 W, 2-minute duration of each workload step. Spiroergometry of three patients was finished prematurely because of reaching the limit blood pressure. The limit of a safe intensity for the training prescription was determined at the level of anaerobic threshold (AT) and expressed in the values of oxygen uptake (VO_{2AT}, VO_{2AT}/kg), heart rate (HR_{AT}) and multiple of metabolic equivalent (METAT). Then all patients were submitted to 12 weeks of walking exercise. To check up the intensity and speed of walking we recommended to the patients the heart rate value at the level of AT and the value of a subjective evaluation of exertion according to Borg scale corresponding to AT level. The training was performed at least three times a week for 30 - 60 minutes. The **final** spiroergometry was made according to the same methodology after 12 weeks of the training programme.

The obtained data were processed by the program Microsoft Excel 97. Statistical analysis was carried out by the Wilcoxon test for pair values at the significance level of 0,05.

RESULTS

The results of the transport system parameters being evaluated are given in Table 1. After the training period the values of HR_{AT}, VO_{2AT}, VO_{2AT}/kg , MET_{AT} and the value of the pulse oxygen PO_{2max} were significantly increased. The increase of the values of W_{max}, HR_{max}, VO_{2max}, VO_{2max}/kg and MET_{max} was insignificant.

Table 1. Results of spiroergometric examination of patients with diabetes before the walking exercise and after it

n = 11	***Before training* (x ± SD)**	***After training* (x ± SD)**	***Statistical* significance**
W_{max} (W)	123 ± 35	127 ± 33	p > 0,05 NS
HR_{AT} (pulse/min)	102 ± 16	108 ± 14	p < 0,05*
HR_{max} (pulse/min)	141 ± 21	145 ± 21	p > 0,05 NS
VO_{2AT} (ml/min)	1079 ± 291	1147 ± 300	p < 0,05*
VO_{2max} (ml/min)	1868 ± 528	1956 ± 509	p > 0,05 NS
VO_{2AT}/kg (ml/min/kg)	12,4 ± 1,8	13,0 ± 1,6	p < 0,05*
VO_{2max}kg (ml/min/kg)	21,3 ± 3,5	22,3 ± 3,1	p > 0,05 NS
PO_{2max} (ml/pulse)	12,9 ± 3,0	13,9 ± 3,2	p < 0,05*
MET_{AT}	3,5 ± 0,5	3,7 ± 0,45	p < 0,05*
MET_{max}	6,1 ± 1,0	6,4 ± 0,9	p > 0,05 NS

Legend
W_{max}- maximum workload, HR_{AT} - heart rate at the level of anaerobic threshold (AT), HR_{max} - maximum heart rate, VO_{2AT} - oxygen uptake at the level of AT, VO_{2max} - maximum oxygen uptake, VO_{2AT}/kg - oxygen uptake at the level of ANP per kg of body weight, VO_{2max}/kg - maximum oxygen uptake per kg of body weight, PO_{2max} - maximum pulse oxygen, MET_{AT}- metabolic equivalent at the level of AT, MET_{max} - maximum metabolic equivalent;
** – statistically significant, NS - statistically insignificant*

DISCUSSION

The studies arranged in a similar way as our study give different results of the training influence on cardiorespiratory parameters in patients with type 2 diabetes. Brandenburg S. I. et al. (10) evaluated the influence of 3 months´ training in a group of women (age 43 ± 7 years, BMI 31,8 ± 6,5) on VO_{2max}., the training being performed three times a week for 50 minutes on a bicycle ergometer in the research centre of the authors with the intensity of 70 - 80 % HR_{max}. The training intensity was checked telemetrically once a week. VO_{2max}

was determined by a direct measurement in the course of spiroergometry (Medgraphics). The authors found out a significant increase of VO_{2max}, did not observe, however, a significant increase of HR_{max} as well as the change of BMI and body weight. They do not discuss the values of HR_{max}, BMI and mass. In our study, on the contrary, we did not find out a significant increase of VO_{2max}, similarly to these authors, however, we observed an insignificant increase of HR_{max}, body mass and BMI. We suppose that the insignificant change of VO_{2max} in our study can be affected by average age of 58 ± 6 years in our group in comparison with 43 ± 7 years of the patients in the above-mentioned study.

The authors Walker K.Z. et al. (8) investigated in their study the influence of 12 weeks´ training programme (60 minutes of uninterrupted walking at least five times a week, intensity degree was chosen by the participants) on VO_{2max}, risk cardiovascular factors and body composition. The group consisted of 11 women with type 2 diabetes (age 58 ± 6 years, BMI 31,1 ± 5,6). VO_{2max} was determined by the method of prediction of VO_{2max} according to Kline et al. (cited according to 8) on the basis of HR determined by walking 1,6 km on the continuous belt, age, sex and body weight. HR_{max} was not evaluated. The authors described a significant increase of VO_{2max} after the 12 weeks´ training programme, with regard to a different method of determination of VO_{2max} in our study (direct measurement), however, our results cannot be compared to those of the authors Walker et al..

Maximum values could be also affected by the premature termination of spiroergometry with three patients because of reaching the limit TK.

CONCLUSIONS

12 weeks´ walking exercise of intensity at the level of anaerobic threshold in patients with type 2 diabetes brought about a significant increase of values of heart rate, oxygen uptake and metabolic equivalent at the level of anaerobic threshold (HR_{AT}, VO_{2AT}, $VO2_{AT}$/kg , MET_{AT}) and of the value of maximum pulse oxygen (PO_{2max}). We consider these changes to be a significant improvement of capacity and economy of the transport system and physical fitness. The training programme with walking being chosen as a physical activity of a low to moderate intensity is tolerated well by the patients; it appears to be safe and applicable in everyday life of motivated patients with type 2 diabetes. Improvement of values at the level of anaerobic threshold can serve as a basis for increasing the training intensity in the next period.

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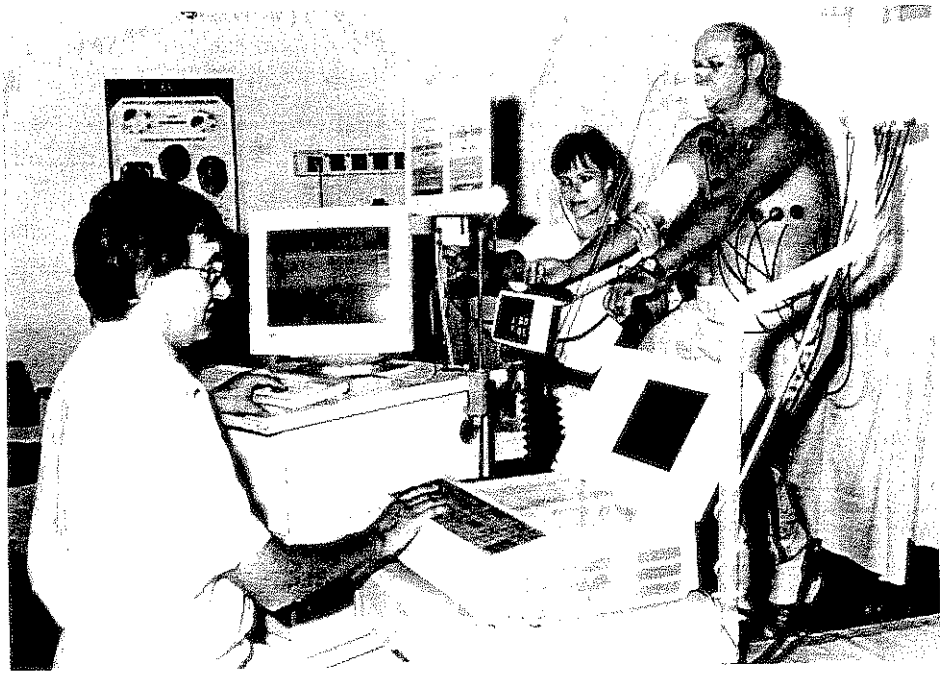
Fig. 1 Spiroergometry



WALKING EXERCISE IN PATIENTS WITH TYPE 2 DIABETES AND BAROREFLEX SENSITIVITY OF HEART RATE

Svačinová Hana, Siegelová Jarmila., Olšovský Jindřich[1], Žáčková Viera[1], Fišer Bohumil[2], Dušek Jiří, Jančík Jiří and Dobšák Petr

Department of Functional Diagnostics and Rehabilitation, [1]IInd Department of Internal Medicine, [2] Department of Physiology, Faculty of Medicine, Masaryk University, Brno

Address correspondence to:

MUDr. Hana Svačinová
Department of Functional Diagnostic and Rehabilitation
Faculty of Medicine, Masaryk University
Pekařská 53
656 91 Brno

INTRODUCTION

Diabetes mellitus and arterial hypertension together with other pathological conditions (insulin resistance, hyperinsulinemia, obesity, dyslipidemia, protrombotic activity of hemocoagulation) are the basic risk factors of aterosclerosis. A complex of these pathological conditions designated as metabolic syndrome of insulin resistance (IR) is accompanied by increased activity of sympathetic nervous system, that is also proved to contribute to the risk of cardiovascular complications (1, 2, 3). Several methods for the determination of the activity of sympathetic nervous system are described, both invasive (microneurography) and non-invasive ones, e.g. determination of heart rate variability (HRV), baroreflex heart rate sensitivity (BRS) and the assessment of heart rate at rest. Depressed values of HRV and a depressed value of BRS indicate the increased sympathetic nervous activity. The ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) study demonstrated that depressed BRS and HRV are strong risk factors for sudden cardiac death in the patients after myocardial infarction. This study and other studies established a significant decrease of the number of surviving patients with a low BRS in comparison with a group of patients with BRS higher than 3 ms/mm Hg (4). In the patients with diabetes the decreased BRS and HRV can be considered to be an early sign of cardiovascular autonomic neuropathy (CAN) representing approximately fivefold increase of the mortality risk. This serious complication of diabetes remains without clinical signs for a long time, that is why detection of its presence is important in its initial stage already (5, 6).

Type 2 diabetes mellitus is accompanied in a majority of patients with developed insulin resistance in the time of manifestation of the disease appearing to be a key factor in the development of other pathological conditions associated in metabolic syndrome. Favourable influencing of IR and of increased sympathetic tone appears to be the main therapeutic objective in patients with type 2 diabetes. Physical activity is also one of non-pharmacological means.

Our study has been concentrated on evaluation of a possibility of affecting favourably baroreflex sensitivity of heart rate in patients with type 2 diabetes by walking exercise.

SUBJECTS AND METHODS

We examined 13 patients altogether, 6 men and 7 women. We formed a group of patients without hypertension (n = 6) and with treated hypertension (n = 7). Basic characteristic of both groups is given in table 1.

Diabetes was treated in all patients by the diet and/or by peroral antidiabetics. No patient manifested specific diabetic complications of a serious degree or other diseases contraindicating the exercise activity being chosen. No patient had clinicalmanifestation of diabetic cardiovascular neuropathy. The participation was determined by a long-term satisfying metabolic compensation; the patients with any form of ischemic heart disease were not included. After the standard internal examination (anamnesis, physical examination, ECG at rest, basic laboratory examination) all patients were subjected to the initial bicycle spiroergometry (Cardiovit CS-10 Schiller, gas analyzer Medgraphics) for the determination of a safe intensity of exercise activity. We chose the protocol with increased workload up to the symptom-limited maximum: basic load 40 W, intensification at 20 W, 2-minute duration of each workload step. Spiroergometry of three patients was finished prematurely because of reaching the limit blood pressure . The limit of a safe intensity for the training prescription was determined at the level of anaerobic threshold (AT) and expressed in the values of oxygen uptake (VO_{2AT}, VO_{2AT}/kg), heart rate (HR_{AT}) and multiple of metabolic equivalent (MET_{AT}). Baroreflex sensitivity of heart rate (BRS) was determined by means of 5 minutes´ continuous recording of blood pressure beat-to-beat (Peňáz method, Finapres Ohmeda) at spontaneous breathing and controlled breathing frequency of 0,33 Hz (breathing according to metronome). The BRS value was determined by spectral analysis of spontaneous fluctuation of systolic blood pressure (SBP) and pulse interval (PI). The value of cross-spectral power density of PI and SBP fluctuation was divided by the value of power spectral density of systolic blood pressure fluctuation at 0,1Hz. The obtained value, modulus was considered to be the measure of BRS. The value of this function at the frequency of 0,1 Hz corresponds to BRS (ms/mm Hg)(Figure 1). Then all patients were submitted to 12 weeks of training by walking. To check up the intensity and speed of walking we recommended to the patients the heart rate value at the AT level and the value of Borg scale corresponding to AT level. The training was performed at least three times a week for 30 - 60 minutes. BRS was determined after 12 weeks of the training programme by the same method.

The obtained data were processed by the program Microsoft Excel 97. Statistical analysis was carried out by the Wilcoxon test for pair values at the significance level of 0,05.

Table 1 Characteristic of subjects

Subjects' groups	n	Age (years) x ± SD	BMI x ± SD	DM duration (years) x ± SD
DM N	6	56 ± 4	30 ± 2	5,3 ± 5
DM H	7	60 ± 8	31 ± 1	12 ± 9

Legend:

DM N - diabetic patients without hypertension; DM H - diabetic patients with treated hypertension

RESULTS

The results of baroreflex sensitivity before the training programme and after 12 weeks of it are given in table 2.

The value of baroreflex sensitivity (BRS) increased after 12 weeks of walking exercise significantly in both groups of diabetic patients. The changes of the values of SBP, DBP and PI in both groups are statistically insignificant.

Table 2. Results of baroreflex sensitivity examination before and after walking exercise

Subjects groups	BRS (ms/mm Hg) x ± SD	SBP (mm Hg) x ± SD	DBP (mm Hg) x ± SD	PI(ms) x ± SD
DM N(1)	3,5 ± 1,1	119 ± 17	71 ± 10	775 ± 114
DM N(2)	4,7 ± 1,2*	122 ± 13	71 ± 13	788 ± 69
DM H(1)	5,1 ± 1,8	140 ± 26	70 ± 14	946 ± 146
DM H(2)	7,2 ± 2,3*	135 ± 18	68 ± 11	944 ± 170

Legend:

*DM N(1) - diabetic patients without hypertension before the training; DM N(2) - diabetic patients without hypertension after the training; DM H(1) - diabetic patients with treated hypertension before the training; DM H(2) - diabetic patients with treated hypertension after the training; BRS - baroreflex sensitivity; SBP - systolic blood ressure; DBP - diastolic blood pressure; PI - pulse interval; * $p < 0,05$ statisticallysignificant change*

DISCUSSION

A favourable influence of the endurance training on autonomous cardiovascular functions was found out both in the experiment with dogs with myocardial ischemia (9) and in our patients with ICHS (10). The authors Howorka et al. (11) found out significant increase of the total spectral power HRV in diabetic patients without any signs of cardiovascular autonomic neuropathy (CAN) and also in diabetic patients with a moderate CAN after 12 weeks of endurance training (65 $\%HR_{max}$, 2 x 30 minutes a week on a bicycle ergometer, age 49,5 years, BMI 25,1). We have also confirmed in our study the hypothesis about the favourable influence

of the training on autonomic cardiovascular functions by using another non-invasive method - determination of BRS. We have also found out that walking as a physical activity of a low - moderate intensity can contribute to increasing the value of baroreflex sensitivity as an indicator of the autonomic nervous system activity. This finding is favourable also because of the fact that walking is easily accessible, safe and psychologically acceptable physical activity for the patients with type 2 diabetes.

CONCLUSIONS

The walking exercise lasting 12 weeks in patients with type 2 diabetes increased significantly baroreflex sensitivity of heart rate. This result supports a hypothesis about the favourable influence of regular physical activity as a non-pharmacological intervention in patients with metabolic syndrome where the simultaneous occurrence of diabetes, arterial hypertension, obesity, dyslipidemia and insulin resistance is accompanied by increased activity of sympathetic nervous system.

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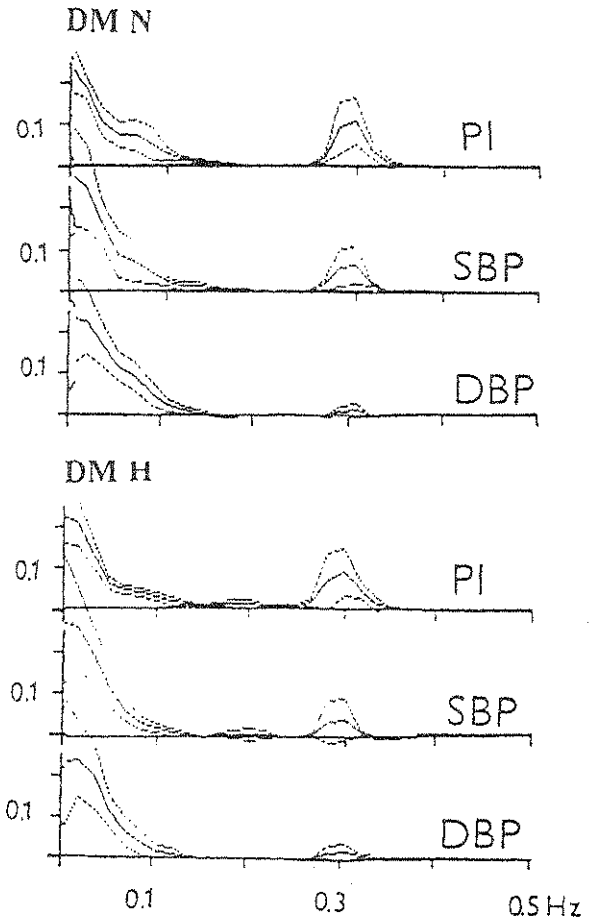

Fig 1a Power spectral density of pulse interval (PI), systolic (SBP) and diastolic (DBP) blood pressure, in normotensive patients with diabetes mellitus type 2 (DM N) and in the hypertensive patients with diabetes mellitus type 2 (DM H

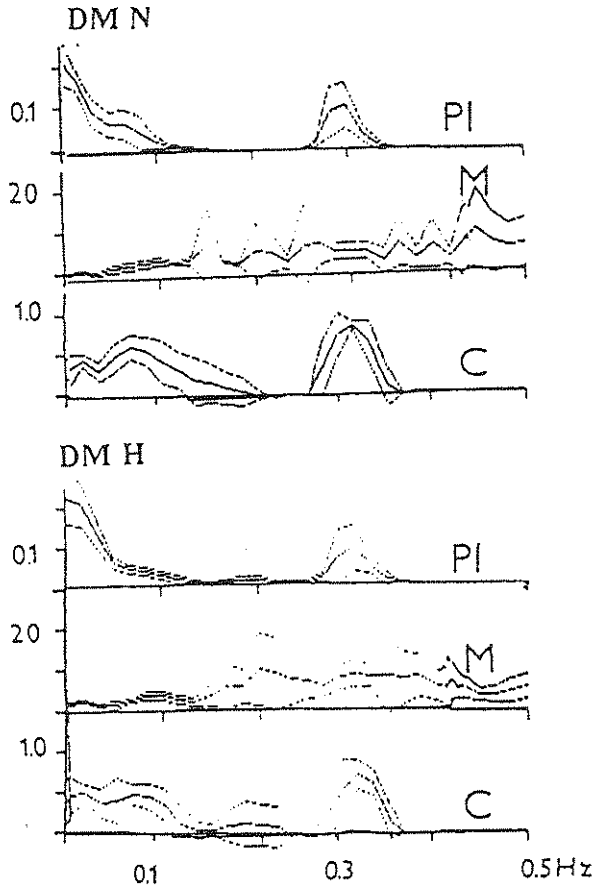

Fig 1b Power spectral density of pulse interval (PI), modulus (M), coherence (C) in the normotensive patients with diabetes mellitus type 2 (DM N) and in the hypertensive patients with diabetes mellitus type 2 (DM H)

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