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Travels with Franz Halberg

Othild Schwartzkopff and Germaine Cornelissen
Halberg Chronobiology Center, University of Minnesota, Minneapolis, MN, USA

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Since the early 1990s, when I renewed my acquaintance with Franz Halberg, I soon joined his team at the laboratory and started participating in ongoing work in chronobiology and chronomics. The Halberg scientific family extended worldwide, and this gave me a chance to become involved in various studies with colleagues from different countries on different continents, most of them revolving around the monitoring of blood pressure and heart rate within the context of a project on the BIOsphere and the COSmos (BIOCOS) coordinated in the Center named after Franz at the University of Minnesota. The pioneering work of Franz earned him numerous invitations to lecture locally, nationally as well as abroad, and this gave me a chance to accompany him on many of his trips and meet in person leading experts who all called themselves Franz's students and were also very dear friends. A few recollections and highlights from these trips are summarized herein.

My first trip with Franz was in May 1996 when we participated in a workshop in Göttingen, Germany, close to my birthplace. There, findings on a partly endogenous biologic week and half-week were discussed. Among those present, Dr. Waldemar Ulmer had provided theoretical evidence for it from the perspective of a physicist [1, 2]. Soon thereafter, in September 1996, we were in St Petersburg, Russia, where the 1st International Congress on Problems of the Noosphere and Sustainable Development took place. A year later, in July 1997, we returned to St Petersburg, Russia, where the XXXIII International Congress of the International Union of Physiological Sciences took place. The occasion was the celebration of a century of achievement in the physiological sciences. The meeting set the scene for the big challenges of physiological science in the 21st century, seeking to integrate knowledge of molecular and cellular processes into an understanding of whole systems. A resolution was formally proposed

concerning the project BIOCOS, an extension of an ongoing womb-to-tomb study, with immediate spin-offs for healthcare and space research. There, we met Professor George Katinas who had been working with Franz in Minnesota earlier. As discussed at an earlier meeting in Ekaterinburg, Russia, with Franz and Germaine, George had brought some programs he had developed for the analysis of chronobiological data. Soon thereafter, Franz arranged for George to return to Minnesota where he spent several years before returning home where he continues his decade-long series of around-the-clock ambulatory monitoring of blood pressure and heart rate [3], as some of us do in Minnesota and Japan [4].

Just a month later, in August 1997, we were in Mexico City, Mexico, where Franz had been invited by his former fellow, Salvador Sanchez de la Pena, to lecture at the II Curso Latinoamericano de Cronobiologia. In September 1997, we were on the road again, this time to Brno, Czech Republic, upon invitation from Professor Jarmila Siegelova. There was already a long-lasting cooperation between Brno and Minneapolis, ever since Professor Pavel Prikryl had invited Franz and Germaine in April 1990 to lecture at an International Symposium on Hypertension. At that meeting, Jarmila and Germaine designed their first joint study to optimize the timing of low-dose aspirin [5]. Many more were to follow, as were yearly meetings on Non-invasive Cardiology, held at Masaryk University. When it became harder for Franz and me to travel, we still participated via Skype.

In May 1998, we were in Vinnitsa, Ukraine, where Franz gave the main lecture at the II International Congress on Integrative Anthropology. It was an occasion to meet with Professor Boris Nikityuk who had obtained records on anthropometric measurements at birth for over 100 years, revealing the presence of about 20-year components [6]. On the way back home, we stopped in Oldenburg, Greifswald, Niemegk and Marburg, Germany, where Franz gave invited lectures and participated in scientific discussions. After giving an invited lecture at the NASA Ames Research Center, in Moffett Field, California, in 1998, a position paper was prepared with Dan Holley on chronoastrobiology [7], the start of a concerted effort to document solar signatures in biological data that eventually led to the concept of congruence and coperiodisms [8-10].

In October 1999, an International Symposium on the Endocrinology of Aging brought us to Tempe, Arizona. We were met there by Dr. Manfred Herold who had published with Franz earlier, documenting the circadian stage dependence of ACTH on urinary cortisol in patients with rheumatoid arthritis [11]. At the time of the meeting, we were cooperating on neuropeptide chronomics [12, 13] after circulating endothelin was found to be characterized by an 8-hour rather than a 24-hour component [14].

June 2000 found us on the island of Crete, Greece, where Franz lectured at the NATO Advanced Study Institute on Space Storms and Space Weather Hazards on "Solar modulations of physiology, pathology and even morphology, and a broader chronoastrobiology". In the proceedings of that meeting, an about 11-year cycle in mortality from myocardial infarction in Minnesota was published, which may be attributed in part to a decreased heart rate variability in the presence of magnetic storms [15]. The next month, Franz was in Germany, where he lectured on stroke prevention. At the Höhenried clinic in Bernried, he was pleased to see Dr. Max Halhuber, former chief physician. His trust bore fruit thanks to Thomas Müller-Bohn who could locate subjects Franz had studied three decades earlier during his rehabilitation after a heart attack: as in other outcome studies, a diagnosis of CHAT (Circadian Hyper-Amplitude-Tension, a condition characterized by an excessive circadian amplitude of blood pressure) was associated with an increased risk of overall mortality [16].

November 2000 saw Franz, Germaine and me in Japan, where we were invited by Professor Kuniaki Otsuka. In Tokyo, we lectured at the 1st International Symposium on Chronoastrobiology and Chronotherapy, a series of 5 meetings organized by Kuniaki [17-21]. After lectures in Sapporo, we all headed to Urausu, where we met the citizens participating in a still ongoing study on stroke prevention, implemented by Kuniaki there and in Tosa City.

I was with Franz in Hanoi, Vietnam, in August 2001, where we participated at the Joint Scientific Assembly of the International Association of Geomagnetism and Aeronomy and the International

Association of Seismology and Physics of the Earth's Interior. There, we met Dr. Christine Amory-Mazaudier, who came to visit us in Minnesota and invited Franz and Germaine to another meeting in Vienna, Austria in April 2007.

On 7 March 2002, Franz and Germaine gave presentations to a small group of local engineers at the Sheraton Four Points Hotel in Minneapolis, Minnesota. They presented results on blood pressure, documenting the need to collect data automatically around the clock for longer than 24 hours, analyzed chronobiologically and interpreted in the light of time-specified reference values qualified by gender and age in order to get a reliable diagnosis. After the presentations, the lead engineer asked a simple question: how can we help? This was the start of our ongoing "Phoenix" Project [22] with a study group of the Twin Cities chapter of the Institute of Electrical and Electronics Engineers aimed at developing an ambulatory blood pressure monitor for the Halberg Chronobiology Center at the University of Minnesota. Its goal is to make a monitor that is inexpensive, unobtrusive and easy to use and collects a week of blood pressure measurements [22].

In April 2002, our trio traveled to Bratislava, Slovak Republic, to participate at an International Symposium on Coenzyme Q10 in biology and medicine. There, we met Dr. Anna Gvozdjakova, who later came to visit us in Minnesota and invited us to contribute a chapter in her book [23]. From Bratislava, we traveled to Kosice, Slovakia, courtesy of an invitation from Professor RB Singh and Prof Daniel Pella to participate at the 2nd International Congress on Cardiovascular Diseases. This meeting provided an opportunity for comprehensive overviews of up-to-the-minute research developments in cardiovascular medicine, particularly regarding atherosclerosis, coronary artery disease and hypertension.

November 2002 found all three of us together with quite a few other BIOCOS members in Munich, Germany, where Professor Dr. Dr. h.c. Theodor Hellbrügge had organized a special meeting to honor Franz with the prestigious Arnold-Lucius-Gesell-Prize. Proceedings of his International Interdisciplinary Conference on "Time Structures -- Chronomes -- in Child Development" were published by Dr. Peter G. Fedor-Freybergh in his journal, Neuroendocrinology Letters [24].

In February 2003, we traveled to India, with a first stop in Hong Kong where Franz gave grand rounds at the Chinese University. In Lucknow, Franz delivered an opening address at the International Conference on the Role of Free Radicals and Antioxidants in Health and Disease, also giving grand rounds at the Department of Medicine at Chhatrapati Shahuji Maharaj Medical University in Lucknow, followed by an invited address at BPCON 2003, a national conference on blood pressure. In Moradabad, upon invitation by Professor RB Singh, Franz lectured at the Moradabad Medical Association, and at the International College of Nutrition. More lectures followed at the Government Hospital and College in Chandigarh, and at the Medical College in Amritsar.

As a pediatrician, I was particularly pleased to have a chance to participate at the X International Congress on Auxology in Florence, Italy, in July 2004. The meeting was organized to present up-to-date information on the various aspects of growth, including the diagnosis and therapy of developmental disorders; the relations between growth and social, educational, environmental, and psychological factors; the study of models of growth, and the secular trend; the study of foetal growth, and progress in the field of genetics. This was also a wonderful opportunity to be with very dear friends with whom Franz had worked for many years. The monograph that was eventually published [25] brought us another friend from Hungary who later published Franz's autobiography [26]. Franz and Germaine left Florence for L'Aquila where they met with Professor Giancarlo Pantaleoni, who, as a student, had attended chronobiologic training at facilities in L'Aquila built specifically for chronobiologic research [27].

November 2004 saw us in Taipei, Taiwan, again courtesy of Professor RB Singh and Prof Chee-Oon Kong. This time, it was for lectures at the 3rd International Congress on Cardiovascular Diseases. We were joined by the late Salvador Sanchez who presented his results on the pre-metabolic syndrome [28], and by other BIOCOS friends. There, we met with Professor Chen-Huan Chen who had sent us his invaluable database of ambulatory blood pressure records, validating results from Kuniaki's outcome study [29].

In January 2005, we were back in India. Franz gave invited lectures at the Jaslok Hospital and at the APICON (Association of Physicians of India Conference) in Mumbai, before going to Raipur to deliver more invited lectures at the Alumni Association of the School of Life Sciences at the Pandit Ravishankar Shukla University and MATS University. The highlight of this trip was the dedication and grand opening of the Halberg Hospital and Research Institute in Moradabad by Dr Mrs Sushav Singh and Dr R B Singh (Figure 1) [30].

Halberg Hospital and Research Institute in Moradabad, India.

Franz Halberg is cutting the ribbon.

Figure 1. Dedication and grand opening of the Halberg Hospital and Research Institute in Moradabad, India.

In February 2006, we went to the Pennington Biomedical Research Center at Louisiana State University in Baton Rouge, Louisiana, where Franz lectured. This visit intensified our cooperation with Professor Weihong Pan and initiated a series of studies with Dr. Alok Gupta who added supportive evidence for a pre-metabolic syndrome [31].

Later that year in September, we traveled to Chengdu, China, by invitation from Professor Zhengrong Wang who had trained with Franz in Minnesota, after Germaine and Franz met him at another conference, also in Chengdu in 1988 organized by Dr. Jinyi Wu. The 2006 International Conference on “Frontiers of Biomedical Science: Chronobiology” included quite a few presentations dealing with blood pressure and heart rate monitoring, emphasizing both clinical applications and basic science in terms of influences from space-terrestrial weather on physiology and pathology.

One month later, we were on the road to Japan for a series of lectures in Tokyo, in Tosa City, and Urausu, by invitation from Professor Kuniaki Otsuka. Staff from the A&D Company which provided the blood pressure monitors for the study accompanied us on the trip.

In August 2007, by invitation from Professor Paul Gomes, we traveled to Lisbon, Portugal, where Franz gave invited presentations at the 56th session of the International Statistical Institute. From early on, Franz realized the need for a rigorous statistical basis without which chronobiology could not have flourished, and he himself developed methodology for chronobiological analyses, earning him several prestigious invitations to statistical meetings such as this one.

In September 2007, we were in Baku, Azerbaijan, where Franz gave invited presentations at the International Academy of Science symposium on "Natural cataclysms and global problems of modern civilization". From there, we traveled to Sudak, Ukraine, for lectures at the VII International Crimean Conference on the "Cosmos and biosphere".

In October 2007, we were back in Japan, where Franz had been invited to lecture at the Nishinomiya-Yukawa International and Interdisciplinary Symposium 2007 “What is Life?” This Hideki Yukawa 100-year Memorial Seminar envisioned “The

Next 100 Years of Yukawa's Dream". The meeting took place at the Yukawa Institute for Theoretical Physics at Kyoto University. Both Franz [32] and Kuniaki [33] delivered major presentations.

We made several trips to Moscow, Russia, where the Halberg Chronobiology Center cooperates with Professor Sergey Chibisov from the People's Friendship University of Russia in Moscow. In 2005, Franz received an honorary doctorate from this University, and Sergey joined BIOCOS to monitor some of his students there. In December 2009, we participated at the 10th International Congress "Health and education millennium: Innovative technologies in biology and medicine" organized by the Network of Young Doctors and Health Administrators.

In October 2010, Franz, Germaine and I were invited to Saudi Arabia to lecture in Al Ahsa at the Third International Conference on Advanced Cardiac Sciences "King of Organs 2010". Lectures during the day were complemented in the evening to trips into the desert. Our gracious host, Dr. Abdullah al-Abdulgader shares our dream to better understand influences from our cosmos on living matter and an environmental geophysical monitoring station had just been completed for this purpose [34].

In November 2010, Franz and I were in Krems, Austria, to lecture at a meeting on "Mozart and Science" by invitation from Dr. Vera Brandes, who later came to visit us in Minnesota. We also met again in September 2011 in Istanbul at the World Forum on Natural Cataclysms and Global Problems of the Modern Civilization, organized by Elchin Khalilov, where Franz served as honorary chairman. Among the several presentations made there, Yoshihiko Watanabe showed that systolic blood pressure started to increase 2 days prior to the 2011 East Japan earthquake [35]. This was the last trip we took together, except for participation to meetings via Skype or by sending a video presentation to be projected at the conference.

As evidenced above, we had a "full" life, and for that, I thank you, Franz. I shall miss you.

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Timing of Statin Therapy May Eliminate Its Toxicity and Increase Its Bioactivity, Said Professor Halberg, The Lord of Time

Teodora Handjieva-Darlenska[1,2], Ram B Singh[*,1,2], Krasimira Hristova[3], Branislav Milovanovic[4], Jan Fedacko[5], Sergey Chibisov[6], Sergey Shastun[6], Vicky Beeharry[6], Svetosslav Handjievi[7], and Chee Jeong Kim[8]

[1]Assistant Professor in the Department of Pharmacology, Medical University in Sofia, Bulgaria
[2]Halberg Hospital and Research Institute, Moradabad, India
[3]University National Heart Hospital, Department of Noninvasive Functional Diagnostic, Sofia, 1000, Bulgaria
[4]President, Neurocardiological Society, University Clinical Hospital, Belgrade, Serbia
[5]Department of Medicine, PJ Safaric University, Kosice, Slovakia
[6]Departments of Physiology and Pathology, People's Friendship University of Russia, Moscow, Russian Federation
[7]Bulgarian Society of Health, Nutrition and Foods, Sofia, Bulgaria
[8]Department of Internal Medicine, College of Medicine, Chung Ang University, Seoul, Korea

Abstract

Halberg, the Lord of Time proposed that any adverse effects of any therapeutic agent can be eliminated or reduced in extent and bioactivity can be increased by the rescheduling the treatment along the 24-hour scale. The treatment may be modifications of routine activities, diet and/or daily exercise and/or statin or antihypertensive medication. For any non-drug or drug treatment the rescheduling in kind and/or timing of administration being gauged by hours after the habitual awakening time or by other marker rhythms such as continuous ambulatory blood pressure monitoring (C-ABPM), wrist activity or a human metabolite timetable. Cholesterol synthesis has a diurnal variation and most of it is synthesized in the night. There is evidence that statins are potent and effective agents with several pleiotropic effects for treatment of hypercholesterolemia and coronary artery disease (CAD). Statins can also decrease sympathetic activity. Statin may have adverse effects, if given in higher doses and in combinations, indicating that it may be a two edged sword. However, if the approach based on timing is used, the dosage of statins may be lowered to achieve greater therapeutic benefit without having any adverse effects of the drug.

Keywords: Statin toxicity, inflammation, drug therapy, chronotherapy

Background

The Lord of Time, Franz Halberg demonstrated that adverse effects of given drug can be eliminated or reduced in extent by the rescheduling along the 24-hour scale [1-10]. This approach can be used for any of non-drug or drug treatment such as modifications of routine activities, diet and/or daily exercise and/or of statin medication. The rescheduling in kind and/or timing of administration being gauged by hours after the habitual awakening time, or by other marker

[*] **Correspondence:** Prof. Dr Ram B Singh, MD, FICC. Halberg Hospital and Research Institute, Civil Lines, Moradabad (UP)244001, India, Email: rbs@tsimtsoum.net

rhythms, such as C-ABPM, wrist activity or a human metabolite timetable [3-10].

Statins are amazing drugs influencing wide range of physiological, biochemical and biological functions [11,12]. This list includes hypolipidemic, vasodilative, antithrombotic, antioxidant, anti-inflammatory, antiproliferative, anticoagulant, agiogenic and bone formation inducing functions. Statins also decrease sympathetic activity and the bioavalability of statins may not translate in to bioactivity if given at wrong time. Myopathy is the most frequent side effect of statins and in some cases may have a form of severe rhabdomyolysis [13,14]. Less common adverse effects include hepatotoxicity, peripheral neuropathy, impaired myocardial contractility and autoimmune diseases. Rare manifestations of statin intolerance may be; pulmonary, psychiatric, ophthalmic and amyotropic lateral sclerosis. The spectrum of statin-related myopathy ranges from common but clinically benign myalgia to rare but life-threatening rhabdomyolysis. Thus statin, appears to be a two edged sword having both; beneficial and adverse effects [15]. The risk of adverse effects is largely outweighed by great reduction of cardiovascular events in statin users by decreasing LDL cholesterol and C-Reactive proteins [16,17]. Clinical evidence suggest that myalgia can occur in up to 10% of subjects prescribed statins, whereas rhabdomyolysis continues to be rare. The mechanisms of statin-related myopathy are unclear and the criteria of diagnosis of myopathy do not consider the symptoms of patients in absence of raised muscle enzymes [13,14].

Periodicity in Cholesterol Synthesis and Statin Use

The rate of cholesterol synthesis in the liver shows diurnal periodicity, with production peaking at night time. Statins act by inhibiting the enzyme HMG CoA reductase, which controls synthesis of cholesterol in the liver. Statins are recommended that they should be taken at night, on the basis of physiological studies which show that most cholesterol is synthesized when dietary intake is at its lowest [18-21]. A small study using simvastatin reported significant differences in total cholesterol and LDL when comparing simvastatin use in the morning and at night time [19]. This study showed that simvastatin, which has a short elimination half-life, taken in the evening instead of the morning decreases the total cholesterol and LDL cholesterol levels more significantly and that alternating times of simvastatin ingestion does not change HDL cholesterol or triglyceride levels. There was a 7.3% higher total cholesterol and a 13.4% increase in LDL when the ingestion time was in the morning compared to the patient previously taking medication at nighttime. However, a systematic review concluded that the evidence base supports evening administation of simvastatin [20]. The review pointed out a statistically nonsignificant trend in the LDL-C percentage reduction favouring evening statin administration with lovastatin, pravastatin, and rosuvastatin [20]. Atorvastatin (a longer elimination half-life than simvastatin) demonstrated similar LDL-C reduction regardless of administration time. It was concluded that there are sufficient data to support evening administration of simvastatin to achieve optimal lowering of LDL-C levels. However, there is insufficient evidence to support nightime or evening administration of the other statins included in the review. A fluvastatin study revealed that the efficacy and safety profiles of fluvastatin XL are equivalent for morning and evening administration [21]. It is possible that the elimination half-lives of atorvastatin, pravastatin, pitavastatin and rosuvastatin are significantly longer than that of simvastatin and this is probably significant in the ability to take these other statins in the morning with non-significant changes in lipid lowering compared to evening administration. (Table 1).

When to Administer Statins

Further long term follow up studies should be examined to find out incidence of adverse effects of statins with evening verses morning administration of statin. These studies did not consider the time of awakening while treatment with statins was administered in the patients, which may be due to ignorance. It is possible that statin therapy if given, according to time structure for example; immediately after awakening, 4 hours, 8 hours or 12 hours after

awakening may provide differences in efficacy and bioactivity as well as in incidence of adverse effects. We need to find out the circadian activity of a biological factor such as cytokine or cholesterol around 24 hour scale and administer the drug according to its maximal level in the 24 hour scale. Coenzyme Q10 may be considered in prophylaxis because a decrease in CoQ in the muscle in presence of toxicity has been observed in several studies indicating, a reduction in serum levels. Chronotherapy with statins as suggested by Halberg may be used to decrease its therapeutic dosage and increase its efficacy in the prevention of cardiovascular diseases. Phamacokinetics of statins are summarized in table 2.

Table 1. Half-lives of statins in the body after ingestion

Statin	Half life
Simvastatin	< 5 hours
Atorvastatin	14 hours
Rosuvastatin	19 hours
Pitavastatin	11 hours

Table 2. Pharmaticokinetic variables of statins

Variable	Pitavastatin	Atorvastatin	Fluvastatin	Lovastatin	Pravastatin	Rosuvastatin	Simvastatin
Prodrug	No	No	No	Yes	No	No	Yes
Lipophilcity (log P)	1.49	4.06	3.24	4.30	−0.23	0.13	4.68
T_{max}	0.5–0.8	1.0–2.0	<1.0	2.0–4.0	1.0–1.5	3.0–5.0	4.0
T 1/2 (h)	11	14	<3	2	2	19	1.4–3.0
Absorption (%)	80	30	98	30	3.4	40–60	60–80
Bioavailability (%)	60	14	24	<5	17	20	<5
Protein binding (%)	96	>98	98	>95	50	88	95
Major P450 metabolic enzyme	CYP2C9	CYP3A4	CYP2C9 (minor)	CYP3A4	None	CPY2C (minor)	CPY3A4
Systemic active metabolites (n)	No	Yes (2)	No	Yes (4)	No	Minimal	Yes (3)
Renal excretion (%)	2	<2	<6	10	20	10	13

Note: This table lists the most important pharmacokinetic variables for all available statins including pitavastatin.

The Importance of Statins for Prevention

We need statin therapy right after birth and infancy although many voices in the public and the medical community argue strongly against the wide spread use of statins for the primary prevention of atherosclerotic cardiovascular disease [22]. The main concerns are about adverse effects, lack of a total mortality benefit, cost, and a philosophical aversion to drug therapy. However, now there is sufficient evidence to refute each of these concerns [23-25]. Recent meta-analyses provide extensive evidence that statins reduce cardiovascular events and total mortality in individuals at lower risk of cardiovascular events than has previously been appreciated, and do so with an excellent margin of safety [23-25]. These studies indicate that of 18 primary prevention statin trials including **56,934** participants, statins significantly reduce all-cause mortality (−14%), fatal and nonfatal cardiovascular disease (−22%), CAD (−27%), stroke (−22%), and coronary revascularization (−38%). These risk reduction benefits occurred in the absence of an increased risk of cancer, myalgia, rhabdomyolysis, liver enzyme

elevation, renal dysfunction, or arthritis but the last endpoint revascularization continue to be open to bias. Moreover, this analysis also included JUPITER trial in which case fatality was greater in the intervention group compared to control group [16,17]. If statins are administered according to time structure, the benefit could be increased and adverse effects can be further minimized. The new-onset diabetes observed to occur frequently in the statin group, consistent with another meta-analysis of statin trials, can also be reduced if statins are administered when beta cells of pancreas are highly active [26].

The recent AHA/ACC cholesterol guideline was the result of a rigorous systematic review of higher-quality randomized trials, and systematic reviews and meta-analyses of randomized trials of drug therapy to reduce atherosclerotic cardiovascular disease events [27]. The evidence provided by the 2013 Cochrane meta-analysis was reviewed as part for the recently released 2013 American College of Cardiology and American Heart Association guideline on the treatment of blood cholesterol to prevent athero-sclerotic cardiovascular disease in adults [24, 27]. The high prevalence of poor lifestyle behaviors leading to elevated cardiovascular disease risk factors persists, with myocardial infarction and stroke remaining the leading causes of death in the world. The efficacy of exercise and diet as chronotherapy has been demonstrated to be highly beneficial if taken in the morning after awakening. This has been possible due to the great mind of our great scholar Franz Halberg, the Lord of Time [28-30]

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Effect of Examination on the Circadian Structure of ECG Parameters

Lyazzat Gumarova[1,2], Franz Halberg[1], and Germaine Cornelissen[*1]

[1]Halberg Chronobiology Center, University of Minnesota, Minneapolis, MN, USA
[2]Al-Farabi Kazakh National University, Almaty, Kazakhstan

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Abstract

Seasonal rhythms are an important tool for an organism to adapt to the environment. As part of the problem of adjustment to a load ("stress"), infradian (including seasonal) biorhythms deserve special attention, notably in a climate with a strong contrast between summer and winter. Evolutionary circannual rhythms contribute to the survival of individuals, species and populations of different animals in the face of seasonal changes in habitat. The changing of seasons imposes great demands on organisms, especially in the continental climate of Kazakhstan. This investigation examines any influence of seasons on the daily dynamics of some ECG parameters (notably endpoints of heart rate variability, HRV) in association with the load of an examination in this Central Asian region. All exams were conducted in the morning (at 09:00). Circadian rhythm characteristics of ECG endpoints were compared between days with vs. without an exam, when exams were taken either during winter or summer. In summer, on days with an examination, 24-hour means of SDNNidx and rMSSD (HRV parameters) were decreased, more so at night, when the parameters SDNNidx, rMSSD, pNN50, pNN100, and pNN200 undergo statistically significant changes. Changes of lesser extent were observed in winter. In both seasons, the load of an examination was associated with a shift in the circadian acrophase (phase of maximum of cosine curve approximating the data) that was more pronounced in winter than in summer. On days with an examination, the harmonic content increased, components with a frequency higher than 1 cycle per day (ultradians) accounting for a larger proportion of the overall variance.

Responses of blood pressure (BP) and heart rate (HR) to a load can vary greatly as a function of the circadian stage when the stimulus is applied. Recently, cycles other than circadian and circannual have also been reported, notably components with periods of about 5 and 16 months, detected in longitudinal BP and HR records as well as in mortality statistics from myocardial infarction and sudden cardiac death in different geographic locations. Whether the response to a load such as an examination is also characterized by such non-photic infradians (with a frequency lower than 1 cycle per day) deserves further investigation.

[*] Address for Correspondence: Germaine Cornelissen. Halberg Chronobiology Center. University of Minnesota – MMC8609. 420 Delaware Street SE. Minneapolis, MN 55455, USA. Tel: 651-624-6976, Fax: 651-624-9989
Email: corne001@umn.edu
Website:. http://www. msi. umn. edu/~halberg/

Keywords: blood pressure, circadian, circannual, ECG, Heart Rate Variability (HRV), season, stress.

Introduction

"Stress" (or rather load) is one of the most important health problems in modern society today, being at the same time an integral part of our lives. The ability of an individual to adapt to loads, gauged by success within society and by critical achievements in work, education and sports, depends strongly on their adaptive capacity at the right time. Effects of loads are largely circadian periodic [1]. Research on shift-work also showed the disparity of responses upon presentation of loads at different times of the day [2]. The importance of circannual rhythms ("seasonal variation") is evidenced from the large predictable changes in the incidence of major diseases, notably from cardiovascular causes [3, 4]. Changes in the circadian response to a load, however, have not been extensively studied as a function of when during the year it is administered. This investigation aims at assessing any circannual differences in the circadian rhythm characteristics of ECG endpoints as they are affected by the load of an examination.

Seasonal rhythms are an important tool for an organism to adapt to the environment. As part of the problem of adjustment to a load ("stress"), infradian (including seasonal) rhythms deserve special attention, notably in a climate with a strong contrast between winter and summer. Evolutionary biological rhythms contribute to the survival of individuals, species and populations of different animals in the face of seasonal and daily changes in habitat.

Subjects and Methods

Seven apparently healthy student volunteers aged 21-35 years residing in the city of Almaty (Kazakhstan) was investigated. Each subject contributed a 24-hour record of the electrocardiogram (ECG) using a 3-channel ECG (SHILLER MT-200 HOLTER-EKG V 2.10, Switzerland). This Holter system uses bipolar leads (one positive and one negative diversion) for each channel. Channel 1 corresponds approximately to the modified abstraction V5, channel 2 corresponds approximately to V2, and Channel 3 to V3. Subjects followed their normal lifestyle without limitations in their daily activity during the monitoring session, which was carried out before, during and after the examination, invariably taken from 09:00 to 12:00. Monitoring was carried out on a day without examination and on a day with examination. The same protocol was followed by the same students once during the summer and once during the winter. HRV endpoints were assessed during consecutive 5-minute intervals. They included the magnitude of RR (ms) and the time intervals restricted to normal QRS complexes (NN-intervals). In addition, hourly values of the maximal, minimal and average HR were analyzed.

Each data series was analyzed by cosinor [5-7]. A 24-hour cosine curve was fitted by least squares to the data of each student during each 24-hour recording session, yielding estimates of the MESOR (Midline-Estimating Statistic Of Rhythm, a rhythm-adjusted mean), double amplitude (a measure of the extent of predictable change within a day), and acrophase (a measure of the timing of overall high values recurring each day). Results from the seven subjects were further summarized by population-mean cosinor, separately for control days and for examination days in each season. Parameter tests [8] were carried out to determine whether there were any differences in the circadian rhythm characteristics between summer and winter, and/or between the control day and the day of examination.

Hourly average HR data were also analyzed by least-squares spectra in the frequency range of 1-10 cycles per day in order to determine whether the 24-hour rhythm was invariably the best-fitting component or whether the examination disturbed the circadian rhythm to such an extent that a harmonic term became more prominent than the 24-hour component.

Because the examination lasted only a fraction of the 24-hour span, an effect on circadian rhythm characteristics may not be large enough to be detected, even though an effect better localized around the time of the examination may be present. For this reason, the hourly HR values of each subject on the day of examination were expressed as a percentage of the HR value at the corresponding

clock-hour on the control day, separately for each season. Paired t-tests were applied at each clock-hour to determine whether, across all subjects, there were any differences localized in time, and if so, when did these effects take place.

Results

Most HRV endpoints remained stable. Only SDNNidx and rMSSD showed a statistically significant decrease during the summer session. In summer, SDNNidx (the average of standard deviations of N-to-N intervals for each 5-minute interval), reflecting the integrated effects of sympathetic and parasympathetic divisions of the autonomic nervous system (ANS), averages 70.9 ± 4.5 ms and decreases during the examination to 59.9 ± 4.2 ms. In winter, during the examination, SDNNidx is barely changed and is even slightly (numerically) increased.

Similar results are found for rMSSD (the root mean square successive difference of N-to-N intervals), an indicator of the activity of para-sympathetic autonomic regulation. In the summer, it is reduced during the day of examination (daytime: $P = 0.028$; nighttime: $P = 0.018$), but in the winter, there are no statistically significant changes. A comparison of HRV endpoints shows a statistically significant change associated with the examination in 5 of the 11 HRV indices (SDNNidx, rMSSD, pNN50, pNN100, pNN200).

The average value of all intervals between successive normal QRS complexes (NN) is reduced during the day of examination, but the difference is not statistically significant. In the summer, NN drops from 714 ± 17 to 669 ± 37 ms during the day of examination, and in the winter from 691 ± 29 to 679 ± 29 ms.

SDNN, which reflects the overall tone of the ANS, does not change statistically significantly. SDANN also does not change statistically significantly between the control day and the examination day.

Nightly values of pNN50 (the proportion of over 50 ms intervals divided by the total number of NNs) are higher than the daytime values ($P<0.05$ for both control day and summer session, but not statistically significantly for the winter session). During the summer session, nightly values were significantly reduced from 29.3 ± 3.9 to 17.6 ± 3.7 in association with the exam. In the summer, pNN100% was also statistically significantly reduced at night (from 5.9 ± 1.6 to 2.5 ± 0.6). These changes demonstrate an increased sympathetic coordination which suppresses the activity of an autonomous circuit in association with a load.

Thus, the load of an examination was associated with a statistically significant decrease in rMSSD and SDNNidx in summer, the changes being more pronounced at night, when important changes affect a larger number of parameters (SDNNidx, rMSSD, pNN50, pNN100, pNN200). Changes associated with the examination are more pronounced in summer than in winter. These results are in keeping with earlier findings in rats [9].

In winter, the number of completed QRS complexes is sharply increased on the exam day by comparison with the control day, starting about 2 hours before the test and lasting for the first two hours during the examination. By contrast, in summer, the increase observed on the exam day vs. the control day is of lesser extent, but starts earlier, about 3 to 4 hours before the test, and lasts longer.

Maximal HR values are also increased between 07:00 and 09:00 on the examination day by comparison to the control day in winter. Only a slight elevation is observed in summer, seen primarily around 05:00 and 06:00. Minimal HR values are increased on the exam day from 09:00 to 10:00 by comparison to the control day in winter. In summer, the elevation is much less, but it is present (at least numerically if not statistically significantly) during the entire 24-hour span.

Hourly averages of HR are characterized by a prominent circadian rhythm. In summer, the 24-hour component is statistically significant ($P<0.01$) for all 7 subjects during the control day. During the day of examination, the circadian rhythm is significant ($P<0.001$) in 6 of the 7 subjects, an 8-hour component ($P<0.001$) being the most prominent in the least-squares spectrum of the other subject. In winter, on the control day, the 24-hour component is invariably statistically significant ($P<0.01$) for all 7 subjects, but on the day of examination, the 24-hour component is detected with statistical significance in only 4 of the 6

subjects (P<0.001 for 3 subjects; P=0.02 for the other subject), being of borderline statistical significance (P=0.057 for another subject and not significant for the remaining subject. Moreover, the 24-hour rhythm was the most prominent component in the least-squares spectrum for only 3 of the 6 subjects.

On a population basis, the circadian rhythm is statistically significant on the control days in summer and in winter (P<0.001). On the day of examination, the circadian rhythm was statistically significant in summer (P=0.003), but only of borderline statistical significance in winter (P=0.070). Parameter tests did not find any difference in the MESOR or in the circadian amplitude and acrophase of HR between summer and winter, whether the comparison is made between control days or between days of examination.

No difference is found between the control day and the day of examination in summer either. By contrast, in winter, the 24-hour acrophase is advanced by about 2.5 hours on the day of examination by comparison to the control day (P=0.004), Figure 1. Pooling data between the two seasons, a comparison between the control day and the day of examination also finds the 24-hour acrophase to be advanced by about 2 hours in association with the load of an exam (P=0.003). A very small difference in MESOR (P=0.113) stems primarily from a higher HR MESOR during the day of examination vs. the control day during the summer (84.8 vs. 77.0 beats/min), the difference being much smaller in winter (83.6 vs. 81.0 beats/min).

Figure 2 illustrates differences in the circadian pattern of HR between winter and summer during control days. The hourly data during summer were used as reference, and the HR values at the corresponding clock-hours in winter expressed as a percentage of the summer data, separately for each subject. Relative values averaged across all 7 subjects are displayed in Figure 2 with their standard errors (SEs). Differences from 100% determined by paired t-test (not corrected for multiple testing) are found primarily during the night and at 09:00, the scheduled start time of the examination to be administered on a different day.

Figures 3 and 4 illustrate differences in the pattern of HR on the day of examination by comparison with the control day. Again, the hourly data on the day of examination are expressed as a percentage of the corresponding data on the control day, separately for each subject. Relative values averaged across all subjects are displayed with their SEs. Differences from 100% determined by paired t-test (not corrected for multiple testing) are found primarily during the 2-3 hours preceding the examination.

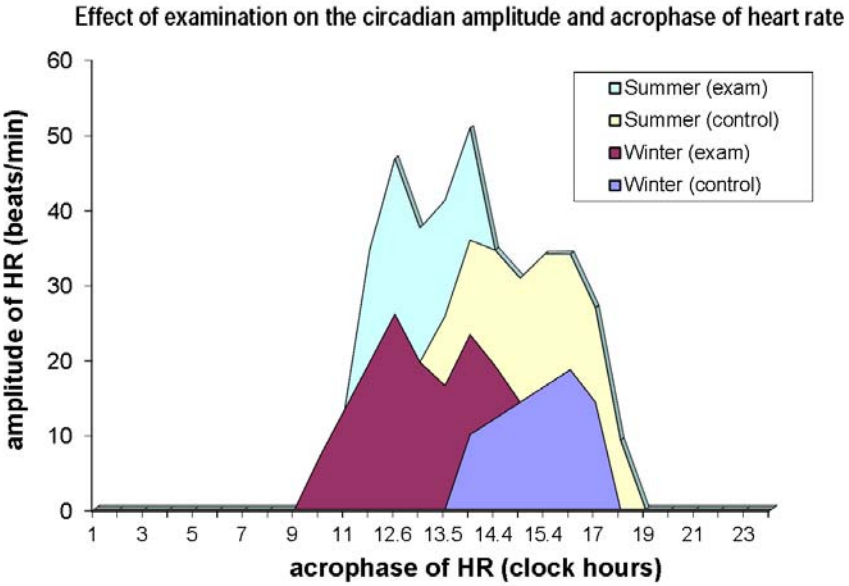

Figure 1. During the day of examination, the circadian acrophase is advanced by 2 to 3 hours. For this analysis, data collected during a day of examination were fitted with a 23-hour rather than a 24-hour cosine curve.

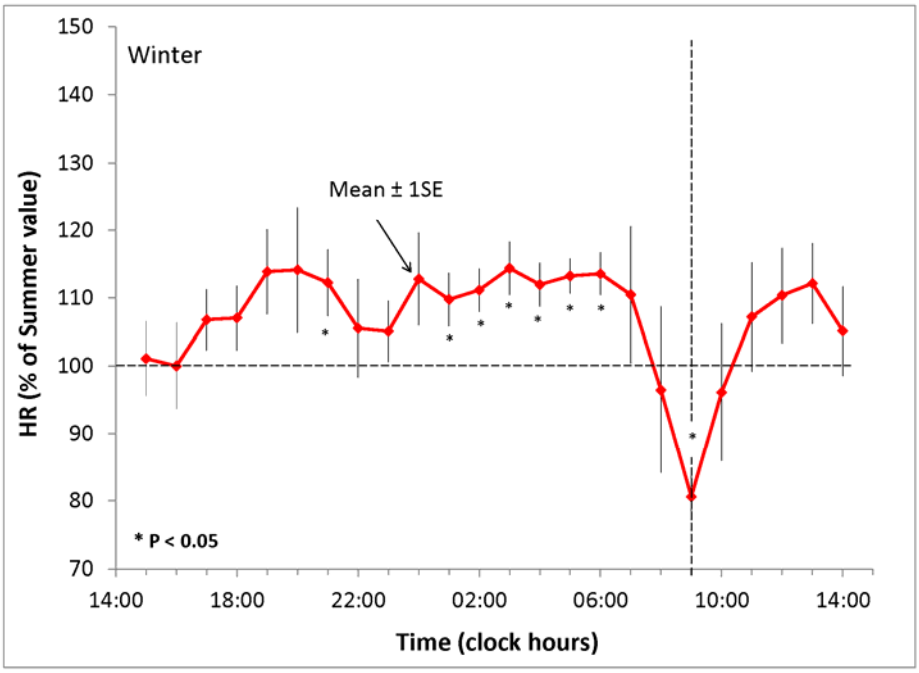

Figure 2. As compared to the circadian profile in summer, HR in winter is slightly higher during the night and lower around 09:00. Data from each subject at each clock hour are expressed as a percentage of the HR value at the corresponding clock hour during summer, and then averaged across all subjects to test for deviation from 100% by paired t test. Data collected during control days in both seasons.

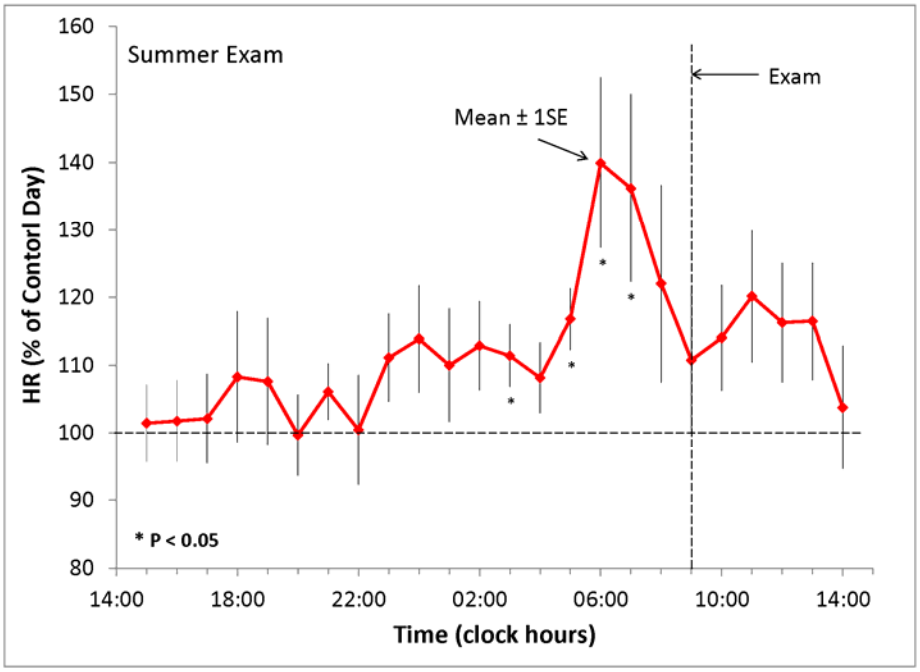

Figure 3. As compared to the circadian profile during the control day, HR on the examination day in summer is higher primarily 2 to 4 hours prior to the test. Data from each subject at each clock hour are expressed as a percentage of the HR value at the corresponding clock hour during the control day in summer, and then averaged across all subjects to test for deviation from 100% by paired t test.

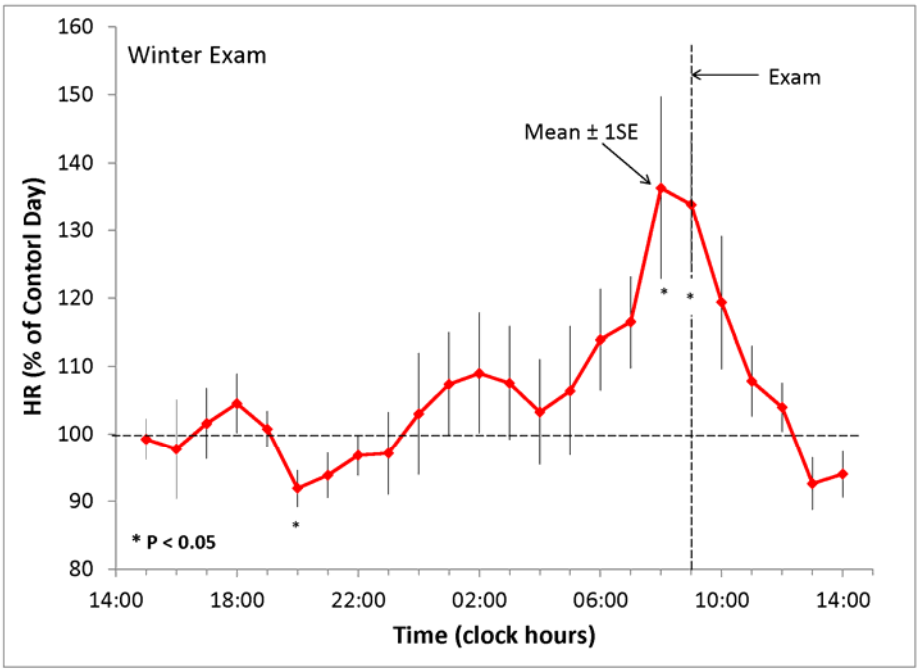

Figure 4. As compared to the circadian profile during the control day, HR on the examination day in winter is higher just before the test. Data from each subject at each clock hour are expressed as a percentage of the HR value at the corresponding clock hour during the control day in winter, and then averaged across all subjects to test for deviation from 100% by paired t test.

Discussion and Conclusion

Short-term responses of the hypothalamic-pituitary-adrenal (HPA) system are very effective in the presence of danger, but at the same time, long-lasting loads consume a marked amount of stored energy needed for winter survival [10], weakening the organism. Photoperiodic coordination of the HPA system can integrate positive and negative effects of stress hormones, as previously shown in studies of the influence of hypokinesia on catecholamines and corticosteroids in rats at different times of the year [11].

The lack of a difference in MESOR of the average HR between the day of examination and the control day indicates that the load was adequate and did not exceed the adaptive capacity of the organism. At the same time, changes in the circadian time structure evidenced by the shift in acrophase and the increased prominence of ultradian components indicate that the load of the examination was associated with a disturbance of the circadian system, in keeping with work by others [12, 13].

The effect of a load depends on the circadian stage when it is administered, notably in relation to the cardiovascular system [1, 14-16], sometimes accounting for differences in opposite direction. In this study, the examination always took place between 09:00 and 12:00. At that circadian stage, the load of an examination had different effects in summer and winter. Differences in 24-hour averages of different HRV endpoints were observed primarily in the summer. By contrast, a difference in the circadian acrophase of HR was most prominent in winter.

Whether these differences between summer and winter are related to seasonal differences reported in the concentration of corticosteroid hormones in peripheral blood and in the weight of adrenal glands and their reactivity to various stimuli [17] deserves further study.

In the experimental laboratory, stressful situations are associated with an increase in corticosterone, and the endocrine response to ACTH (if not to loads) also shows a circadian stage dependence [18-20]. A higher increase in corticosterone in response to ACTH occurs when the hormone is at its circadian minimum,

and a lesser response occurs at the circadian maximum [18-20].

The critical circadian stage dependent response of the cardiovascular system to a load has been extensively documented by Franz Halberg. Herein, we show that the response to an examination taken in summer or winter also makes a difference. Recently, cycles other than circadian and circannual have also been reported, notably components with periods of about 5 and 16 months, detected in longitudinal BP and HR records as well as in mortality statistics from myocardial infarction and sudden cardiac death in different geographic locations [21-24]. Whether the response to a load such as an examination is also characterized by such non-photic infradians (with a frequency lower than 1 cycle per day) deserves further investigation.

In any event, as a minimum, further investigations should carefully record the date and time when a load is applied. Preferably, applying the same load at different rhythm stages rather than at a fixed time is likely to be more informative, notably when the response to periodontal surgery can be an increase in BP and HR when it is performed in the morning, or a decrease when the same procedure is done in the afternoon [15]. Other factors such as physical activity may be confounders with any examination effect, because exercise is asspciated with an increased heart rate variability [25].

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Studies on Circadian Pattern of Blood Pressure in Normotensive Pregnant Women and Preeclampsia. In Memory of Late Professor Franz Halberg, Father of Chronobiology

RK Singh[*1,4], NS Verma[2], Neelam Barnwal[3], HP Gupta[3], Urmila Singh[3], Seema Mehrotra[3], Ranjana Singh[1], and RB Singh [5]

Departments of [1]Biochemistry, [2]Physiology and [3]Obstetrics & Gynecology, King George's Medical University, Lucknow, [4]Department of Biochemistry, SGRRIM & HS, Dehradun, [5]Halberg Hospital and Research Institute, Moradabad, India

Abstract

With the aim of comparing the circadian characteristics of blood pressure (BP) in normotensive pregnant women and preeclampsia, a total of 35 pregnant women with gestational age more than 20 weeks (age: 18-40 years) were recruited from the patients admitted in the Department of Obstetrics & Gynecology, Queen Mary's Hospital, King George's Medical University, Lucknow. In these patients, systolic (S) BP was 140 mmHg or above and diastolic (D) BP was 90 mmHg or above on 2 consecutive occasions, with measurements taken 6 hours apart. Thirty five age-matched pregnant women were diagnosed as 'normotensive', when their casual BP was always below 140/90 mmHg on at least 3 different occasions were included as Controls. We analyzed BP records obtained by ABPM (TM-2430 monitors from the A&D Company, Tokyo, Japan) for 3 days, observing all the required precautions and after getting their informed consent and proper counseling. Compared with normotensive pregnancies, a statistically significant elevation of the circadian rhythm-adjusted mean (MESOR, a rhythm-adjusted average value) of BP was observed in preeclampsia ($p<0.001$ for both SBP and DBP).

Keywords: •Circadian rhythm • Ambulatory blood pressure monitoring • Pregnancy • Normotensive • Preeclampsia.

Introduction

This study builds on the extensive work of Professor Franz Halberg within the scope of blood pressure monitoring during pregnancy. His pioneering work led to important circadian rhythm alterations predictive of adverse outcomes of pregnancy such as pre-eclampsia [1-24]. Hypertensive disorder complicating pregnancies are common and contribute greatly to maternal and fetal /perinatal morbidity and

[*] ***Address for correspondence***: Dr R K Singh, Professor and Head, Biochemistry Department, Shri Guru Ram Rai Institute of Medical & Health Sciences, Dehradun – 248001 (Uttarakhand), INDIA. Phone: +91-135-2522168 O); Fax: +91-135-2522117 Email singhrk23a@hotmail.com

mortality [25]. Hypertension is the most accessible clinical sign of preeclampsia. BP monitoring in preeclampsia is important and it should be very precise. However, BP assessment in pregnant women has relied mostly on a few measurements taken in the physician's office and these measurements may be influenced by external and internal stimuli, among other factors, by the patient's sleeping or waking schedule, physical activity, diet, and emotional state [25-28]. These casual time-unspecified measurements perform poorly and cannot predict the dangerous life threatening hypertensive episodes. They do not help in regularization of dose and timing of antihypertensive medications. Thus, conventional methods are neither sufficient nor precise enough for BP monitoring in preeclampsia. ABPM has the advantage that in addition to the immediate presentation of repeated automatic measurements of BP, the data can readily be analyzed to assess the circadian variation of BP in pregnancy. SBP and DBP are not constant over a 24-hour period [29]. They show the characteristic circadian pattern in most individuals, including nonpregnant and pregnant women in response to internal clock and mental, physical and social activity.

During the night both SBP and DBP drop by about 10-20%. In clinically healthy pregnant women, BP steadily decreases up to the middle of gestation and then increases up to the day of delivery, with final BP values similar to those found early in pregnancy in the same women [30]. For women who developed gestational hypertension or preeclampsia, BP is stable during the first half of pregnancy and then continuously increases until delivery [25]. These predictable patterns of BP variability during pregnancy are somehow independent from the continuous linear increase in maternal weight with gestational age [25].

ABPM addresses many sources of error associated with the conventional blood BP monitoring. The day-night index is very useful for management of preeclampsia and in prediction of dangerous vascular variability disorders. Changes in circadian variation in BP could be used either to predict preeclampsia or to assess its severity in an attempt for early identification of hypertensive disorders of pregnancy for timely prophylactic interventions. Only a few studies have been made on the normal pattern of ABPM in uncomplicated pregnancies [31-35], most of them without comparison with the circadian pattern of BP in complicated pregnancies.

In the present study, an attempt has been made to evaluate and compare the circadian pattern of BP in normotensive pregnant women and in pregnant women with preeclampsia.

Materials and Methods

Subjects

A total of 35 pregnant women with gestational age more than 20 weeks (age: 18-40 years) were recruited from the patients admitted in the Department of Obstetrics & Gynecology, Queen Mary's Hospital, King George's Medical University, Lucknow. In these patients, on 2 consecutive occasions 6 hours apart, SBP was above 140 mmHg and DBP was above 90 mmHg, the critical thresholds for SBP/DBP, as recommended by the World Health Organization [36].

In all selected patients, a detailed history of present pregnancy along with obstetrical history, past history (especially for chronic hypertension, diabetes and endocrinal diseases), family history, and socio-economic history was taken. A detailed clinical examination was done, including general examination, systemic examination and thorough obstetrical abdominal examination. Reference antenatal investigations for hemoglobin, blood group, HBsAg, HIV, VDRL, blood sugar screening (1 hour after 50 g glucose), and complete urine examination were done. Obstetrical ultrasonography, to establish number of fetuses, fetal well-being, gestational age, placental localization, maturity and amniotic fluid and to rule out any congenital anomaly, was also done in all participants. Liver function test, kidney function test, serum uric acid and serum electrolytes were also done as and when indicated. Patients with chronic hypertension, chronic renal disease, hyperthyroidism, trophoblastic disease and disease requiring anti-inflammatory medications were excluded from the study. The Institutional Ethics Committee approved the study. All volunteers signed consent forms before entering the study.

As controls, 35 age-matched pregnant subjects were diagnosed as 'normotensive', when their casual BP was always below 140/90 mmHg on at least 3 different occasions.

Protocol

All subjects included in the study underwent conventional reference BP recording and ambulatory BP recording by using a TM-2430 ambulatory monitor (A&D Company, Tokyo, Japan) for 3 days. During the recording span, women followed their usual diurnal waking and nocturnal resting routine and followed their usual life conditions without any restriction. They were advised to go to bed before 23:00 as far as possible during the span of observation.

The ABPM device was applied to each subject between 9AM and 4 PM. The procedure was explained to all subjects. All required precautions were taken: patients were counseled about risks and benefits and written consent was obtained from each patient. Subjects were eliminated if they had a night resting span of less than 6 hours or more than 12 hours. The BP cuff was worn on the non-dominant arm. Both SBP and DBP of each individual were sampled every 30 minutes during 08:00 and 22:00 and hourly on the hour between 22:00 to 08:00. During the monitoring span, each subject kept a record of her activity cycle, physical activity, emotional state, other external and internal stimuli, possibly affecting BP.

Statistical Analysis

SBP and DBP data were downloaded into a personal computer. Original oscillometric data were edited according to commonly used criteria for the removal of outliers and measurement errors [37-39]. These original oscillometric data from each BP series was first synchronized according to the sleep-wake cycle. After synchronization, BP values were edited for noting missing values and probable artifacts. BP records were analyzed by the cosinor method to evaluate their circadian characteristics. This analysis provides estimates of the parameters of the circadian pattern of blood pressure BP, namely the MESOR (Midline Estimating Statistics of Rhythm), 24-hour Amplitude and Acrophase. In addition, the Hyperbaric Index, and Percentage Time Elevation were determined. The circadian characteristics of the two groups were compared.

Results

35 cases and 35 controls were studied; their reference characteristics are given in Table 1.

Table 1. Reference characteristics of cases and controls

	Controls (n=35)	Cases (n=35)	P value
Age (Mean±SD)	25.81±4.058	26.16±3.611	NS
Primigravida: multigravida	13:22	12:23	NS
Mean gestational age (weeks)	33.86±4.012	35.43±3.12	NS
Body Mass Index (BMI)	22.20±1.976	23.707±2.791	NS
Systolic Blood Pressure	112.97±5.75	146.14±7.75	<0.05
Diastolic Blood Pressure	69.89±6.02	93.26±4.88	<0.05

Table 2. Associated complication in study group

S.No.	Associated Complication	Number of cases (n=35)	Percentage
1.	Intrauterine growth retardation	8	22.86
2.	Oligohydramnios	4	11.43
3.	Anemia	4	11.43
4.	Preterm Labour Pain	4	11.43
5.	Decreased fetal movement	2	5.71
6.	Uncomplicated	13	37.14

Table 3. Circadian characteristics of BP recordings in preeclampsia and controls

Circadian parameters		Controls (n=35)	Patients (n=35)	P-value
MESOR	Systolic BP	109.05± 7.42	137.77±11.10	<0.001
	Diastolic BP	66.35± 5.99	87.77± 8.51	<0.001
Amplitude	Systolic BP	10.19± 4.09	8.40± 2.91	0.058
	Diastolic BP	7.86± 2.64	6.08± 2.14	0.043
Acrophase of Systolic Blood Pressure				
Normal pattern		32 (91.43%)	17 (18%)	
Abnormal (Disrupted pattern)				
Shifted towards Evening		2 (5.71%)	9 (25.71%)	
Shifted towards Night (Reverse pattern)		1 (2.86%)	9 (25.71%)	
Acrophase of Diastolic Blood Pressure				
Normal pattern		32 (91.43%)	18 (51.42%)	
Abnormal (Disrupted pattern)				
Shifted towards Evening		2 (5.71%)	8 (22.86%)	
Shifted towards Night (Reverse pattern)		1 (2.86%)	9 (25.72%)	
Hyperbaric Index				
Systolic Blood Pressure		16.88± 12.19	327.63± 125.51	<0.001
Diastolic Blood Pressure		21.84±10.62	341.63±128.93	<0.001
Percentage Time Elevation				
Systolic Blood Pressure		10.66±6.62	65.44±21.11	<0.001
Diastolic Blood Pressure		14.97±10.82	74.62±20.51	<0.001

Compared with normotensive pregnancies, a statistically significant elevation of the circadian rhythm-adjusted mean (MESOR, the average value of the rhythmic function fitted to the data) of BP is found in preeclampsia ($p<0.001$ for both SBP and DBP). There is also a statistically highly significant difference in the circadian amplitude (difference between the maximum and the MESOR of the fitted curve) of both SBP and DBP between the two groups.

Discussion

In the present study, all cases and controls underwent the conventional BP monitoring as well as ambulatory BP monitoring and BP was continuously recorded for 3 days. The present study confirmed the circadian variability of BP in the two groups.

The circadian pattern of BP in preeclamptic women differs from the circadian pattern of BP in normotensive pregnant women. Highly statistically significant differences in all the parameters of the circadian rhythm of BP (both systolic and diastolic)

have been observed between normotensive and pregnant women with preeclampsia.

The MESOR of SBP and DBP was significantly higher in pregnant women with preeclampsia than in normotensive pregnant women. This reflects that the 24-hour mean of BP is much higher for the preeclamptic group as compared to normotensive pregnant women. These results are in accordance with previous reports [40].

The amplitude of SBP and DBP in the preeclamptic group is less than in normotensive pregnant women. But the difference reaches statistical significance only for DBP while for SBP it is marginally insignificant. Previous studies report similar results with significant differences in SBP and DBP [40, 41].

In the present study, blunting of the circadian pattern (amplitude <7.5 mmHg) was noticed to be more common in preeclamptic women (40% and 65.7% for SBP and DBP, respectively) by comparison to normotensive pregnant women (27.8% and 34.2% for SBP and DBP, respectively). Blunting of circadian rhythm is abnormal and denotes the severity of preeclampsia and increases in BP have already been associated with overt pathology, as in the cases of severe preeclampsia, it is the compensatory mechanism of the body to maintain organ blood flow during sleep in response to organ ischemia which is commonly found in preeclamptic women [42-47]. The acrophase of DBP in preeclamptic women occurred after 18:05 in 48.5% cases; in 25.71% cases, it occurred after 22:00 (i.e., reverse pattern). The reverse pattern has also been reported in other studies with greater or lesser extent. The blunting of the nocturnal drop in BP [31,34,42-45] and a reverse pattern of the circadian rhythm of BP both have important implications if one considers that the cardiovascular system is not able to sustain an excessive load during the night hours, and in clinical practice BP is usually measured at another time of day [32,35,46]. The calculation of the hyperbaric index in each case allowed us to quantify the severity of BP excess over the threshold value and of increased risk of cardiovascular disease. The hyperbaric index represents a better determinant of BP load than the MESOR. A higher hyperbaric index is a proper determinant of BP excess [47, 48]. In pregnancy, the hyperbaric index derived from ambulatory monitoring is markedly superior to office measurements for diagnosis of what should be truly considered gestational hypertension, as well as for prediction of the outcome of pregnancy [49]. In the present study, the hyperbaric index for SBP and DBP was significantly higher in preeclamptic women than in normotensive pregnant women. Moreover, values of maximum HBI below the critical threshold from normotensive pregnant women were consistently associated with uncomplicated pregnancies. These results are in keeping with previous findings [48].

The percentage time elevation helps quantify the total duration of BP excess during 24 hours and is a good measure of the severity of preeclampsia. The percentage time elevation of SBP and DBP is significantly higher in preeclamptic women than in the control group. Our observations are in agreement with previous reports [48].

Ambulatory BP monitoring helps predict preeclampsia and assess its severity in an attempt for early identification of dangerous hypertensive episodes of pregnancy for timely prophylactic interventions, which is not possible by conventional BP monitoring. Ambulatory BP monitors are invaluable in regularization of dose and timing of antihypertensive medication. Deviation from the normal circadian pattern of BP also helps in the prediction of preeclampsia because it develops much before the actual onset of hypertension, which needs further confirmation.

Conflicts of Interest

The authors declare there is no conflict of interest.

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Mitochondrial Cardiomyopathy and Coenzyme Q_{10}

A. Gvozdjáková[1*], M. Mikulecký[2], FL. Crane[3], J. Kucharská[1], G. Cornelissen[4], A. Kumar[5], P. Palacka[7], and RB. Singh[6]

[1]Comenius University in Bratislava, Medical Faculty, Pharmacobiochemical Laboratory of 3rd Department of Internal Medicine, Bratislava, Slovakia
[2]Department of Statistics and Biometry, Neuroendocrinology Letters, Stockholm, Sweden, and Bratislava, Slovakia
[3]Department of Biological Sciences Purdue University, W. Lafayette, Indiana, USA
[4]Halberg Chronobiology Center, University of Minnesota, Minneapolis, USA
[5]Department of Cardiology, Government Medical College/GND Hospital, Amritsar, India
[6]Halberg Hospital and Research Institute, Moradabad, India
[7] 2nd Oncology Department, Medical School, Comenius University and National Cancer Institue, Bratislava, Slovakia

Dedication

This chapter is dedicated to the memory of Prof. Franz Halberg, father of chronobiology in the world (Figure 1). At our Medical Faculty, Prof. Halberg together with Prof. RB Singh and Prof. G Cornelissen presented Halberg's chronobiology method to study mitochondrial heart and brain oxidative phosphorylation (OXPHOS) and coenzyme Q rhythms in experimental medicine, which could be a trigger for acute myocardial infarction and sudden cardiac death. Recently, Prof. Halberg was the author of a chapter on Chronobiology in our book [1].

Using Halberg's method we conducted a pilot study on heart mitochondrial chronobiology. Prof. Mikulecký et al. [2, 3] found circadian rhythms in heart mitochondrial OXPHOS, and derived circadian and circasemidian cascade parameters of OXPHOS and mitochondrial coenzyme Q_{10} – „Q_{10}-CLOCK“.

Figure 1. Professor Franz Halberg.

[*] ***Address for correspondence***: Anna Gvozdjáková, Comenius University in Bratislava, Medical Faculty, Pharma cobiochemical Laboratory of 3rd Department of Internal Medicine, Bratislava, Slovakia, Email: anna.gvozdjakova @fmed.uniba.sk

Abstract

Heart mitochondrial oxidative phosphorylation function and coenzyme Q_{10} (CoQ_{10}) concentration in clinical and experimental cardiomyopathies are presented. In endomyocardial biopsies (EMB) of patients with cardiomyopathy of unknown etiology (CPUE), decreased mitochondrial respiration, ATP production and CoQ_{10} concentrations have been found. A positive relationship between the degree of rejection, decreased CoQ_{10} and OXPHOS function in EMB of transplanted hearts has been documented. In experimental medicine circadian and circasemidian rhythms of heart mitochondrial „CoQ_{10}-CLOCK“ have been found and different parameters of the cascade of oxidative phosphorylation between control and diabetic rats' hearts were estimated. Mapping changes in heart CoQ_{10} and ATP production along the 24-hour scale can help obtain a better understanding of triggers of acute heart attacks. A novel CoQ binding site in Voltage-Dependent Anion Channel (VDAC) of outer mitochondrial membrane is proposed [4]. CoQ_{10} targeted therapy of damaged mitochondria could be in the site of porin (VDAC) of the outer mitochondrial membrane where exogenous CoQ_{10} passes through this channel into mitochondria. Based on current knowledge, target of mitochondrial respiratory chain supplementary therapy with CoQ_{10} in cardiomyopathies is warranted.

Keywords: mitochondrial cardiomyopathy, coenzyme Q_{10}, chronobiology, diabetes

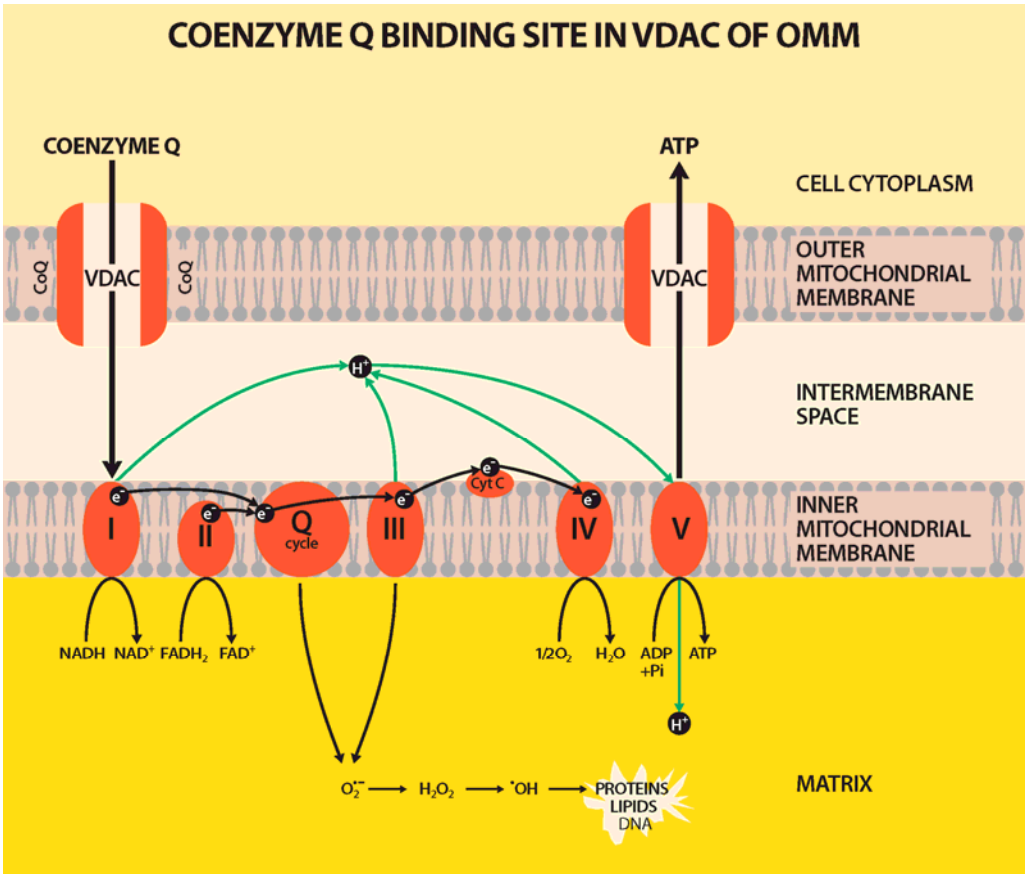
Introduction

Mitochondria are subcellular organelles, which produce almost 90% ATP for myocardium function. Heart physiological function depends mainly on mitochondrial energy production. Mitochondria represent from 20 to 40% of cardiomyocyte volume. Various metabolic mitochondrial pathways are important for heart energy production, such as β-oxidation fatty acids, electron transport respiratory chain as well as coupling of oxidative phosphorylation. A key component of mitochondrial electron respiratory chain is coenzyme Q (CoQ). Its function is in a „Q-CYCLE“in three forms: as ubiquinol (reduced CoQ), ubiquinone (oxidized CoQ) and radicals form (semiquinone). The dominant human form is CoQ_{10}; that in rats is CoQ_9. Mitochondria are a major source of energy production in the heart as well as a source of reactive oxygen species (ROS) and nitrogen oxygen species (NOS) in the cell. Uncontrolled free oxygen radical production participates in many metabolic disturbances and damage of heart function. Low CoQ_{10} concentrations in the heart mitochondria and impaired oxidative phosphorylation (OXPHOS) function are considered to be the molecular basis of heart failure and could participate in triggering acute myocardial infarction, sudden death and stroke.

Mitochondria's architecture consists of four compartments, an outer (OMM) and inner (IMM) mitochondrial membrane, inter-membrane space and the matrix. OMM separates the cytosol from the inter-membrane space. IMM separates the inter-membrane space from the matrix. The folding of the IMM (cristae) serves to increase the surface area of this membrane. Five complexes of the respiratory chain are located in the IMM. Complex I: NADH dehydrogenase-ubiquinone oxidase, Complex II: succinate-dehydrogenase-ubiquinoneoxidase, Complex III: ubiquinone cytochrome c oxidore ductase, Complex IV: cytochrome c oxidase, Complex V: ATP-synthase. Mobile components of the respiratory chain are cytochrome c and coenzyme Q10. Complexes I-IV receive electrons from the catabolism of carbohydrates, fats and proteins and generate a proton gradient across the inner mitochondrial membrane. Complexes I and II collect these electrons and transfer them to coenzyme Q10, Complexes III and IV. Complexes I, III and IV utilize the energy in electron transfer to pump protons across the IMM, producing a proton gradient, which is used by Complex V for adenosine triphosphate (ATP) production from adenosine diphosphate (ADP) and inorganic phosphate. Produced ATP is translocated from the mitochondria into the cytoplasm by adenine nucleotide translocator [5, 6].

As an important component of the respiratory chain, coenzyme Q_{10} has several functions. It transfers electrons and protons between Complexes I and III, and between Complexes II and III. CoQ_{10} participates in mitochondrial ATP production, acts as an antioxidant, and regulates free oxygen radicals production. A novel CoQ function has recently been proposed, wherein the plasma membrane redox function depends on coenzyme Q. This CoQ redox control function includes Voltage Dependent Anion Channel (VDAC) [4, 7]. These authors propose that a novel CoQ function has therapeutic implications for coenzyme Q_{10} treatment in autism and in other neurological diseases. A novel mechanism of CoQ

binding site in VDAC of outer mitochondrial membrane is shown in Figure 2 [8]. In the last few years, mitochondria have been the target of antioxidants and drugs therapy for the regeneration of damaged mitochondria, in various human disorders, such as cardiovascular and neurological diseases, in cancer, infertility, kidney and liver diseases.



Legends: CoQ: Coenzyme Q; VDAC: Voltage Dependent Anion Channel; ATP: adenosine triphosphate; ADP: adenosine diphosphate; Pi: inorganic phosphate; I, II, III, IV, V: respiratory chain complexes; H^+: proton; e^-: electron; Q-cycle: Coenzyme Q cycle; cyt c: cytochrome c; NADH: reduced nicotinadenindinucleotid; NAD^+: nicotinadenindinucleotid; $FADH_2$: reduced flavinadenindinucleotid; FAD: flavinadenindinucleotid; $O_2^{\bullet-}$: superoxide radical; H_2O_2: hydrogen peroxide; $OH^{\bullet}$: hydroxyl radical; H_2O: water; O_2: oxygen.

Figure 2. Coenzyme Q binding site in VDAC of outer mitochondrial membrane.

Mitochondrial Chronobiology of the Heart

Variations as a function of time in heart mitochondrial OXPHOS and CoQ_{10} concentrations could be triggers of myocardial pathobiochemical functions. Singh et al. [9] reported that the incidence of chest pain was highest in the second quarter of the day, concerning 41% of the 202 patients with acute myocardial infarction (AMI) In an Indian study (1978), 39% of 605 patients with acute myocardial infarct had onset between 06:00 and 12:00 hours. In a WHO report from 19 European Centers and the Soviet Union, Singh et al. [10] reported a peak onset incidence of chest pain due to AMI between 8:00 and 11:00.

Most experimental studies were done during the daylight hours. Some published articles focused on biological rhythms of growing yeast [11]. These data suggest a mechanism of respiratory oscillation in

yeast growing in continuous culture involving cycles of energization and de-energization of mitochondria. Ultradian clock-driven cycles of energy demand, slower oscillatory dynamics in comparison with energy transformations in mitochondria, and ultradian clock-driven cycles of energy demand were observed. In isolated rat brain mitochondria, Simon et al. [12] found that the highest respiratory control value (3.01) occurred at 04:00 and the lowest value (2.63) at 08:00. The highest value of state-3 occurred at 12:00 and the lowest at 20:00. Lloyed et al. [13] hypothesized that yeast mitochondrially-generated reactive oxygen metabolites provide long-term cyclic energization of mitochondria, and unrepaired mitochondria eventually lead to cellular senescence and apoptosis, after a number of respiratory cycles have elapsed.

Physiological heart function depends on mitochondrial function of heart cells and their energy production mainly through OXPHOS. Five parameters are used for the evaluation of Complexes I and II of mitochondrial OXPHOS function: (1) ADP:O, coefficient of OXPHOS; (2) OPR, rate of ATP production; (3) S_4, basal respiration; (4) S_3, ADP stimulated respiration; and (5) RCI, respiratory control index. Experimental animals can be used for a study of heart mitochondrial chronobiology. Very little is known about the circadian variation of heart function.

In our previous study, Halberg's antiphasic lighting regimens were used in three-month-old Wistar rats for synchronization during 60 days. One regimen, LD12:12 (light onset at 10:00), was shifted to sample in the dark during waking hours [14]. Halberg's cosinor was used to test for 24- and 12-hour periodicity [15]; Student's t-tests were also applied for statistical evaluation, Figure 3.

Circadian Variations of OXPHOS in Mitochondria of Control Rat's Heart

Circadian variations of OXPHOS show different time courses for Complex I and Complex II of the respiratory chain, with two circadian maxima (at 24:00 and 08:00) and minima at 20:00 of heart mitochondria of control rats, Table 1 [14, 16-18].

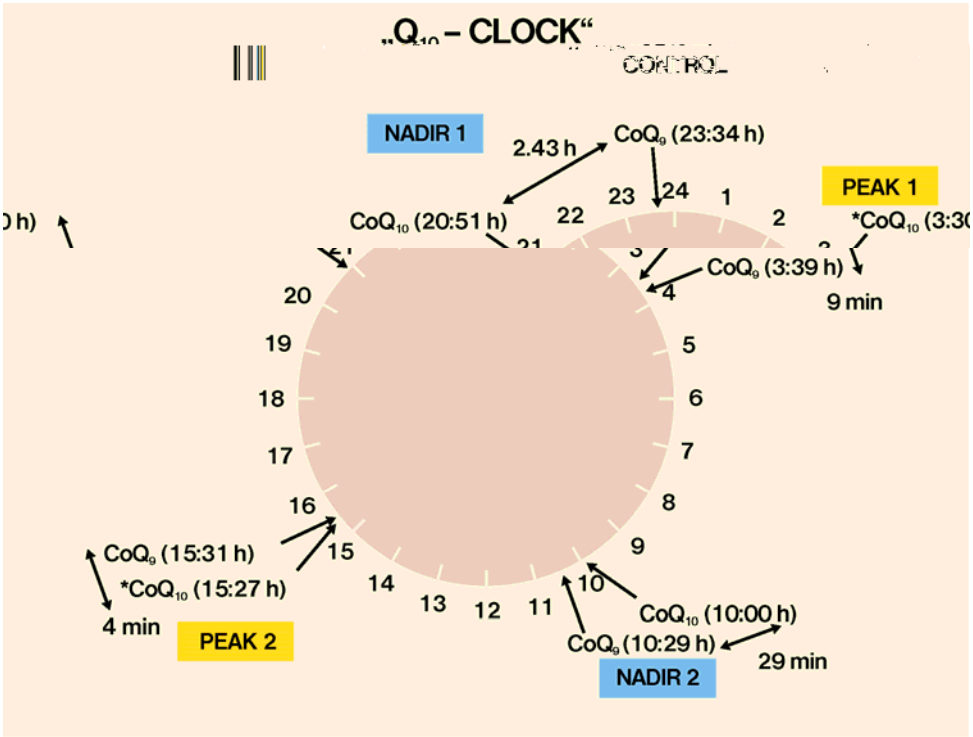


Figure 3. "Q_{10}-CLOCK" in heart mitochondria of control rats.

Table 1. Circadian variations of OXPHOS in mitochondria of control rat's heart

Hours	8:00	12:00	16:00	20:00	24:00:00	4:00
Complex I.						
ADP:O (nmol/nAtO)	3.380	3.139	2.847	2.743	2.951	3.003
OPR (nmol ATP/mg prot.min)	**442.50**	337.90	309.00	**239.00**	**713.80**	355.50
S3 (nAtO/mg prot/min)	**180.70**	141.90	140.80	**112.00**	**277.10**	154.70
S4 (nAtO/mg prot/min)	38.82	27.74	24.65	25-Oct	38.65	24.73
RCI (S3/S4)	Apr-68	May-15	May-71	Apr-44	Jul-28	Jun-30
Complex II.						
ADP:O (nmol/nAtO)	1.781	1.703	1.378	1.534	1.898	1.573
OPR (nmol ATP/mg prot.min)	**372.90**	259.00	247.10	**177.10**	**519.40**	302.00
S3 (nAtO/mg prot/min)	**255.00**	210.10	236.70	**148.20**	**277.20**	251.20
S4 (nAtO/mg prot/min)	163.50	121.60	149.20	94.00	164.15	153.85
RCI (S3/S4)	Jan-56	Jan-73	Jan-57	Jan-57	Jan-81	Jan-65

Table 2. Circadian and circasemidian parameters of cascade of OXPHOS in control rat's heart

		PEAK 1 (24 h) (h)	PEAK 2 (12 h) (h)
Complex I	**S4**	9:02	23:42
	ADP:O	9:16	0:00
	OPR	10:47	0:58
	S3	11:02	1:13
	RCI	14:22	2:14
Complex II	**S4**	10:04	4:33
	ADP:O	10:04	0:11
	OPR	10:14	1:02
	S3	12:18	3:02
	RCI	12:51	6:34

Circadian and Circasemidian Cascade of OXPHOS of Control Rat's Heart

Two PEAKS (maxima) and two NADIRS (minima) were found for a cascade of OXPHOS.

For Complex I: Maximal OXPHOS activity: circadian PEAK 1 (24 hours) was between 09:02 and 14.22 (lasting 5.20 hours), and circasemidian PEAK 2÷ (12 hours) was between 23:42 and 02:14 (lasting 2.5 hours). Minimal OXPHOS activity: circadian NADIR 1 was between 16:21 and 19:31 (lasting 3.10 hours) and NADIR 2 between 00:00 and 05:34 (lasting 5.5 hours). *For Complex II:* Maximal OXPHOS activity: PEAK 1 was between 10:04 and 12:51 (lasting 2.5 hours) and PEAK 2 was between 00:11 and 06:23 (lasting 6.2 hours). Minimal OXPHOS activity: NADIR 1 was between 17:13 and 19:20 (lasting 2.1 hours) and NADIR 2 was between 00:00 and 05:39 (lasting 5.7 hours), Table 2 [3]

Mitochondrial „Q_{10}- CLOCK" of Control Rat's Heart

Circadian and circasemidian components of heart mitochondria were statistically significant only for

CoQ_{10-OX} (and not for CoQ_{9-OX}) of control rats. Maxima (PEAK 1 and 2) were at 03:30 and 15:27. NADIRS of CoQ_{10} were at 20:51 and 10:00 (Table 3). Most circadian rhythms in humans are in antiphase with those of rats.

This pilot study found: (1) a statistically significant circadian cascade of OXPHOS for PEAK 1 at Complex I and Complex II; (2) statistically significant circadian and circasemidian components for CoQ_{10} of rat's heart mitochondria [2, 3].

Summary of maximal circadian OXPHOS activity and maximal circasemidian concentration of CoQ_{10} during: a. *Rats' activity:* Complex I activity lasting 2,32 hours (from 23:42 to 02:14). When Complex I activity decreased, after 1.16 hours, maximal CoQ_{10} concentration was reached (at 03:30). Complex II activity lasted 6.12 hours (from 00:11 to 06:23). During this time, maximal CoQ_{10} concentration was reached (at 03:30). b. *Rats' inactivity*: it is regeneration time of OXPHOS. Complex I activity lasted 5.20 hours (from 09:02 to 14:22). After 1.03 hours (at 15:27), maximal CoQ_{10} concentration was reached. Complex II activity lasted 2.47 hours (from 10:04 to 12:51). After 3.38 hours (at 15:27), maximal CoQ_{10} concentration was reached.

Heart mitochondrial cascade of OXPHOS and changes in „Q_{10}-CLOCK" may play an important role in the pathogenesis of altered heart function and in mitochondrial cardiomyopathies. We suggest that heart mitochondrial circadian and circasemidian variations of coenzyme Q and oxidative phosphorylation may be important in the pathogenesis of altered heart function, Table 3. Mitochondrial „Q_{10}-CLOCK" appears to be a key parameter for the regeneration of mitochondrial membrane and for the re-energization of control rat's heart mitochondria. The mapping of heart mitochondrial CoQ_{10} and ATP production along the 24-hour scale may help the understanding of triggers of acute heart attacks [14, 18].

Table 3. Circadian variations of CoQ_{10} in mitochondria of control rat's heart

Hours	8:00	12:00	16:00	20:00	24:00:00	4:00
Coenzyme Q 10-OX (nmol/mg prot.)	**0.327**	0.469	0.717	0.390	0.688	**0.948**
Coenzyme Q 9-OX (nmol/mg prot.)	**2.290**	4.080	4.520	3.047	5.280	**7.800**

Cardiomyopathy and Coenzyme Q_{10}

The term "cardiomyopathy" was introduced in clinical cardiology in 1957 [19]. According to the WHO classification, cardiomyopathy is the chronic and permanent damage of the heart muscle of unknown etiology without the involvement of the coronary arteries. Cardiomyopathies are: *hypertrophic cardiomyopathy* (muscle hypertrophy and ventricular outflow tract of the adjacent septum, causing an obstacle in the systolic phase with increased hemodynamic gradient between the left ventricle and the aorta; *dilated cardiomyopathy,* dilatation of all four sections of the heart with principal fibrotic changes of myocardium; and *restrictive cardiomyopathy,* characterized by endocardial thickening in the chambers, that significantly reduces their volume. Another group is called *specific myocardial damage*, induced by such known causative agents as alcohol, smoking and drugs [20] or cardio(myo)pathies of unknown etiology [21]. Cardiomyopathies are associated with major CoQ_{10} deficiency. CoQ_{10} administration improves myocardial function, clinical status of patients, physical activities and myocardial mitochondrial function.

Mitochondrial Cardiomyopathy

Mitochondrial cardiomyopathies are characterized as damage of heart mitochondrial function and metabolism, which are included in mitochondrial diseases. The first demonstration of mitochondrial dysfunction in a human skeletal muscle was made by Luft and was termed "Luft's disease" [22]. In isolated mitochondria of striated muscle, an uncoupling of oxidative phosphorylation was found.

The high level of cytochrome c oxidase and reactive low level of CoQ_{10} was evidence of mitochondrial disturbances. Mutation of mtDNA was first documented in neurodegenerative disorders [23].

A primary cause of mitochondrial diseases is a defect in nuclear DNA (nDNA) encoding for mitochondrial protein or in mitochondrial DNA (mtDNA). nDNA describes defects of respiratory chain subunits, mitochondrial motility, transcription or translation. Mutations in mtDNA have been linked to dysfunction of mitochondrial respiratory chain function, decreased ATP production and changes in fatty acid oxidation. Secondary causes of mitochondrial diseases are related to factors such as ischemia, reperfusion, cardiovascular diseases, diabetes, oncologic diseases, alcohol, smoking, drugs, stress and aging.

Mitochondrial cardiomyopathies could be genetically derived or acquired. mtDNA mutations are clinically expressed mostly as various myopathies together with cardiomyopathies. Dilated and hypertrophic cardiomyopathies are generally due to base substitution in mtDNA [24]. Damage of OXPHOS is predominantly seen in genetically-dependent mitochondrial diseases. Mutations of mtDNA, with damage in OXPHOS, were identified in more than thirty of thirty-seven genes. Cardiomyopathies are often associated with defects in mitochondrial respiratory chain function and ATP production, CoQ_{10} deficit and increased membrane permeability. Cardiomyopathy associated with diabetes, smoking, and alcohol consumption and ischemia-reperfusion injury can be included in acquired mitochondrial cardiomyopathies [25]. The clinical picture of patients with mitochondrial cardiomyopathy includes hypertrophy of both chambers, their significant prolongation and cardiac failure within 4 weeks. In biopsy samples, an extension of the muscle fibers with the rest of myofibrils and an increased presence of fat fraction were found. Mitochondrial disorders were in bc_1-COX complex and multiple disorders in respiratory chain. Inherited damage of Complex I [26] and disorders in Complexes I and II of respiratory chain were reported in patients with KMP in EMB [27].

Clinical Medicine

Mitochondria are essential subcellular organelles in every eukaryotic cell. Mitochondrial oxidative phosphorylation produces almost 90% of energy in the cell, necessary for the continual heart function. They synthesize heme, lipids, amino acids, and nucleotides and maintain homeostasis of inorganic ions. Mitochondria contain 5-10% of cellular proteins, which are imported into mitochondria from the cytoplasm and represent the main mechanism of mitochondria biogenesis [28]. While most oxygen consumed by mitochondria is reduced to water at complex IV, about 1 to 2% of oxygen acquires electron directly, generating reactive oxygen radicals (ROS), as superoxide ions, converted to H_2O_2 and hydroxyl radical. Uncontrolled excessive production of ROS provokes megamitochondria formation. Mitochondria become enlarged with lowering of the rate of oxygen consumption and energy production [29]. Leakage of ROS may lead to damage of the mitochondrial membrane, proteins, and mtDNA [30, 31].

Changed function of myocardium mitochondria was not included in mitochondrial diseases for a long time. Damages of the heart muscle mitochondria were found in diseases of brain and skeletal muscle - in mitochondrial myopathy or encephalomyopathy. Disorders of mitochondria in mitochondrial cardiomyopathy include damage in OXPHOS (NADH-CoQ reductase, CoQ-bound proteins, cytochrome c oxidase, cytochromes bc_1 and cyt aa_3, failure adeninenuckleotide translocator, ATP-ase), disturbances in the synthesis of enzymes, dehydrogenases (long chain acyl dehydrogenase CoA, pyruvate DH, alpha-keto-glutarate-DH), carnitine cycle and creatine kinase system [32]. Mitochondrial respiratory chain function and energy production in endomyocardial biopsies of patients with cardiomyopathy of unknown etiology and in patients after heart transplantation is shown below [33] in part 5.1.1. and 5.1.2.

Heart mitochondrial OXPHOS function in EMB of patients waiting for heart transplantation (CPUE), CoQ_{10} and alpha-tocopherol

Cardio(myo)pathies of unknown etiology (CPUE) constitute a group of diseases with cardiac symptoms

and/or findings that neither the clinical, laboratory nor comprehensive noninvasive cardiac tests can even include in the generally accepted diagnostic category. *Inclusive criteria:* patients with blood pressure: <160/90 mmHg, age: 18-60 years, classification of NYHA: II-IV, BMI <30. NYHA classification was used for groups, as NYHA II: 5 patients, NYHA III: 5 patients, NYHA IV: 2 patients, NYHA II(A): 2 patients dependent on alcohol. *Exclusive criteria:* Ppatients with ICHS, acquired or congenital heart disease, arteriovenous shunts, pulmonary hypertension, known pericardial disease, including myocardial and endocardial right ventricular dysplasia, hypertrophic CMP, intracardiac departments and serious diseases extracardial diseases [21]. According to the applied method by Veksler et al. [34], skinned fibers were prepared for the study of bioenergetic and respiratory properties of mitochondria in human endomyocardial biopsies of patients awaiting heart transplantation as well as after heart transplantation [35]. Baseline (V_0) and stimulated mitochondrial respiration (V_{ADP}) and cytochrome oxidase activity in EMB is showed in Table 4. For measurement of Complex I, NAD-linked substrate (glutamate) was used; for Complex II, FAD-linked substrate (succinate with inhibitor rotenone) was used. In patients dependent on alcohol reference mitochondrial respiration at Complex I as well as cytochrome c oxidase activity were increased in comparison with non-alcohol patients. Stimulated basal mitochondrial respiration and increased Complex IV activity could be adaptation mechanism against damaged myocardium mitochondria, Table 4 [35].

Table 4. Mitochondrial respiration, energy production and cytochrome c oxidase in EMB of CPUP patients

NYHA	Complex I		Complex II		Complex IV	
	V_{ADP}	Cytochrome c oxidase		Cytochrome V_0	V_{ADP}	V_0
	6.00	Jun-80	6.00	Sep-80	**NYHA II.**	8.00
)	Feb-40	Mar-20	4.00	8.00	**NYHA III.**	Feb-80
5	4.00	8.00	11-Oct	10-Oct	**NYHA IV.**	Mar-95
	Jun-20	10-Oct	13-Oct	**37.50**	**NYHA II (A)**	**15.70**

CoQ_{10} and alpha-tocopherol concentration was increased in NYHA III (T) and CoQ_{10} in blood and EMB concentration in NYHA IV (A) patients in comparison with patients without tumor and alcohol dependence, Table 5. Stimulated CoQ_{10} production in tumor and alcohol heart EMB and blood of CPUE patients could be defence and adaptation of organism against damaged heart mitochondria and oxidative stress, increased lipid peroxidation in the plasma of patients [36].

Heart mitochondrial OXPHOS function in EMB of patients after heart transplantation and CoQ_{10}

The discovery of cyclosporin A, an immunesuppressive drug, is important for the extent of successful transplantation of vitally important organs in clinical medicine. On the one hand, it has been used as immunesuppressive therapy, and on the other hand, it has side effects. Cyclosporin A is hepatotoxic, even in very low concentrations, it damages mitochondrial functions, inhibits mito-chondrial permeability transition pore, characterized by progressive permeabilization of the inner mito-chondrial membrane, stimulated by osmotic support [37]. Heart transplantation is an accepted therapy for patients with end-stage heart failure. Years of patient survival after heart transplantation depend on various factors, such as number of rejections, immune-suppression, free radicals production, antioxidant systems function, mitochondrial respiratory chain function and ATP production, as well as coenzyme Q_{10} and carnitine concentration in the heart. Patients with a transplanted heart require continual complex medical care and therapy for a lifetime. They have to be prevented of infection diseases.

Table 5. CoQ_{10} in blood and EMB, and α-tocopherol in plasma and EMB of CPUP patients

NYHA		CoQ10		α-tocopherol	
		Blood (μg/ml)	EMB (μg/g ww)	Plasma (μg/ml)	EMB (μg/g ww)
	mean				
NYHA II	(n=3)	0.278	44.00	Oct-81	619.00
NYHA III (T)	(n=1)	**0.312**	**122.00**	**16.40**	**3790.00**
NYHA IV (A)	(n=1)	**1.480**	**288.00**	Apr-13	164.00

T = right ventricle tumor
A = alcoholic cardiomypathy
n = number of patients

Infections represent major complications for patients with a transplanted heart. Inflammatory processes, activated by neutrophils, which participate in uncontrolled free radical production are involved in the mechanisms of acute rejection of a heart transplant [38]. The role of stress proteins and their correlation with the degree of cellular rejection of the human transplanted heart was first documented by Moliterno et al. [39].

Acute rejection in the first year after heart transplantation is one of the greatest problems. Although histological evaluation of EMB is one of the standard diagnostic methods for detection of the early symptoms of rejection of the transplanted heart, we tried to find new mitochondrial biomarkers of early rejection symptoms of a transplanted heart. Diminished CoQ_{10} concentration in EMB of these patients were related to the degree of rejection of transplanted hearts (Figure 4). We found a correlation between endogenous myocardial CoQ_{10} concentrations and the degree of rejection of the human transplanted heart [40, 41].

Twenty-eight EMB were divided according to histologically confirmed degree of rejection; 0 = without rejection; 0-1 = incipient rejection; 1= mild rejection; 2 = moderate rejection. Mean age of patients was 45 years, range 16-63 years, patients were 1-9 years after heart transplantation. The mean of CoQ_{10} concentration in EMB in heart transplanted patients was 36.7±3.72 μg/g ww. The results were statistically evaluated in comparison with the group of patients without rejection. In the incipient rejection group, CoQ_{10} concentration was statistically significantly decreased (35.9± 5.19 μg/g ww vs. 54.9± 7.79 μg/g ww, $P<0.05$). In rejection 1, CoQ_{10} in EMB was 26.6 ± 4.65 μg/g ww, $P<0.01$). In rejection 2, content of CoQ_{10} in EMB was 25.2 ± 8.74 μg/g ww, $P<0.05$. This methodological approach for early and rapid determination of rejection development, focusing on mitochondrial respiratory chain function and energy production in EMB of heart transplanted patients, was used. Baseline (V_1) and stimulated (V_{ADP}) mitochondrial respiration in EMB of heart transplanted patients relate to the degree of rejection, using NAD- and FAD- linked substrates, Figures 5 and 6. Damaged mitochondrial respiration and ATP production at the site of Complex I are observed in degree rejection 1. The FAD- site of respiratory chain was more sensitive to rejection development, with respiration inhibited already in degree 0 - 1 rejection. Damage of mitochondrial respiration and energy production at the site of Complex II was also found in relation to rejection development of transplanted heart (Figures 5 and 6).

Decreased mitochondrial energy production and coenzyme Q_{10} concentration could be involved in the pathobiochemical mechanisms of the acute rejection development of the human transplanted heart [33, 40-43]. Decreased CoQ_{10} concentration in the transplanted heart correlates with decreasing mitochondrial energy production and rejection episode development. Supplementary therapy for patients with transplanted heart with CoQ_{10} is thus warranted.

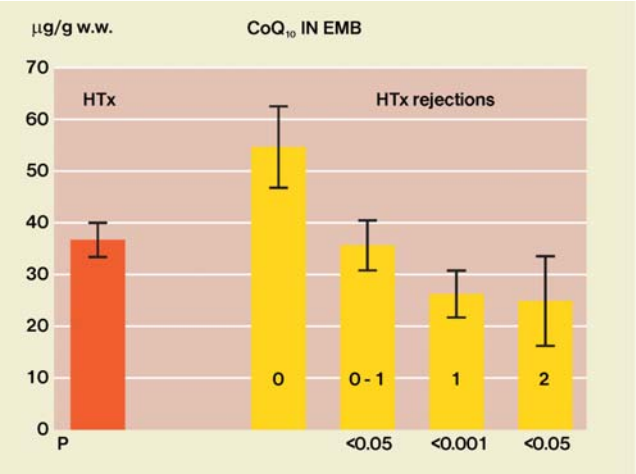


Figure 4. Relationship between coenzyme Q_{10} concentration in EMB and degree of rejection of human transplanted heart.

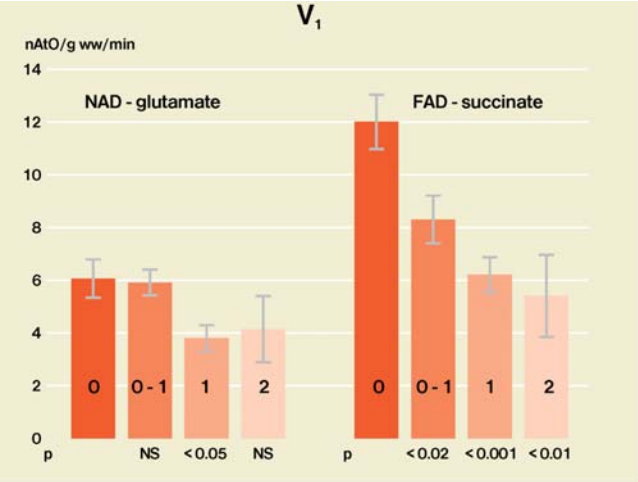


Figure 5. Baseline mitochondrial respiration (V_1) in EMB of heart transplanted patients in relation to the degree of rejection of human transplanted heart.

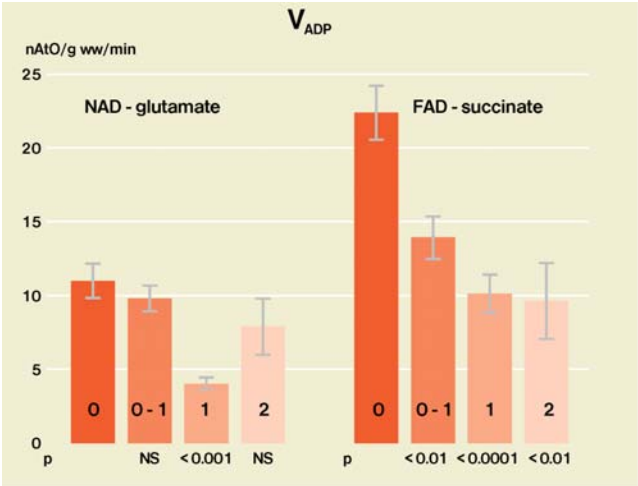


Figure 6. Mitochondrial ATP production (V_{ADP}) in EMB of heart transplanted patients in relation to the degree of rejection of human transplanted heart.

Conclusions: In pathobiochemical mechanisms of rejection of the transplanted human heart, bioenergetic processes of heart muscle mitochondria are also involved. Diminished CoQ_{10} concentrations are directly associated with mitochondrial respiration and bioenergetics of the heart muscle. The concentration of CoQ_{10} limits function of the respiratory chain at the Complex II site. This methodology could be used as a new mitochondrial biomarker of early rejection symptoms of heart transplantation. For confirmation of these results, a further study of heart mitochondrial function and CoQ_{10} concentration estimation in EBM of heart transplanted patients is warranted. Treatment of these patients with CoQ_{10} could improve heart mitochondrial function, support the antioxidant defence system, and thus participate in the prevention of rejection of the transplanted heart.

Diabetic Cardiomyopathy and CoQ_{10}

Diabetes mellitus (DM) is a chronic disease caused by impairment of insulin secretion in pancreatic β-cells. Insulin lowers blood glucose by stimulation of glucose transport to muscle and fat cells and inhibition of its generation in the liver. Impaired secretion of insulin by β-cells as well as loss of its action (insulin resistance) in peripheral tissues results in hyperglycemia. Glycation is intimately associated with enhanced free radicals production. Oxidative stress based on the uncontrolled production of free oxygen radicals and decreased endogenous antioxidants contributes to the degenerative changes in pancreatic β-cells [31] and participates in the development of chronic diabetic complications including diabetic cardiomyopathy, neuropathy and angiopathy [44]. Many other diseases, such as cardiomyopathy, myopathy, encephalomyopathy, renal failure, visual failure, stroke, seizures and dementia, can accompany DM.

DM classifications based on etiology include: DM Type 1 (characterized by β-cells destruction), DM Type 2 (resistance to insulin action), mitochondrial diabetes (caused by mitochondrial DNA defect) and gestational diabetes [45]. DM is a major problem not only from a medical point of view, but also economically. Across the planet, DM currently affects more than 150 million individuals, and it is expected to more than double by the year 2025 [46]. The average life expectancy of subjects with DM diagnosed before 40 years shall be reduced by 11 years. In the age group over 65 years, DM incidence is 10-fold higher compared with the over 45-year group. Prevalence of coronary heart disease in diabetics is 2-14 times higher than in non-diabetics [47].

In our previous study, patients with diabetic cardiomyopathy (DCMP) were on supplementary therapy with CoQ_{10}, α-lipoic acid (ALA) and vitamin E during three months. *CoQ_{10}* or vitamin Q is produced in every cell; it is essential for ATP synthesis and participates in many metabolic pathways. The highest CoQ_{10} concentration is in organs that require the highest energy: heart, liver and brain [48]. CoQ_{10} has been considered for improving glycemic control, acting as antioxidant [49]. *ALA* has been shown to be beneficial in oxidative stress, ischemia-reperfusion injury, diabetes, cataract formation, and neurodegeneration [50]. In patients with DM, ALA concentrations are depleted, resulting in neuropathies. A beneficial effect of ALA was shown in ALADIN studies and improved cardiac function was demonstrated [51]. *Vitamin E* is responsible for protecting against PUFA in membranes and lipoproteins against peroxidation by scavenging peroxyl radicals and breaking chain propagation steps. Acting as a scavenger of free oxygen and lipid peroxyl radicals, alpha-tocopherol is oxidized to tocopheryl radical. Active alpha-tocopherol can be regenerated by ascorbic acid or ubiquinol. Tocopheryl radical can be recycled in mitochondrial electron transport chain. Vitamin E has a potential protective role against chronic disease processes. Chronic simultaneous effect of CoQ_{10}, ALA and vitamin E on selected echocardiographic parameters, oxidative stress and antioxidant in plasma of patients with DCMP was evaluated [52, 53]. In this study 19 patients were included (12 men, average age 61.6±9.0 and 7 women, 63.3±9.0 years). They had DM-Type2 and DCMP, were overweight (BMI 29±6.3), and had average blood sugar values of 7.62±1.06 mmol/L, with less well-controlled hypertension (systolic blood pressure: 140.5±6.5 torr; diastolic blood pressure: 85±14.1 torr) and cardiothoracic index values of 0.46±0.05; they were treated with insulin (n=10) and/or oral antidiabetic drugs (n=12).

Supplementary therapy was given in two daily doses (60 mg hydrosoluble CoQ_{10}, 100 mg ALA and 200 mg vitamin E) during 3 months. All parameters were statistically evaluated by a paired Student t-test (before start of the study and after 3 months of supplementary therapy): glycemia, glycosylated haemoglobin (HbA_1C), CoQ_{10}, vitamin E concentrations, lipids peroxidation (malondialdehyde, MDA) and selected echocardiographic parameters of left ventricular (LV) systolic and diastolic function: EF, ejection fraction; LVESD and LVEDD, end-systolic and end-diastolic LV diameters; IVS, intraventricular septum thickness; PW, LV posterior wall thickness; DT, deceleration time, and E/A, early velocity/velocity during atrial systole ratio (Table 6).

Table 6. Simultaneous effect of CoQ_{10}, ALA and vitamin E in patients with diabetic cardiomyopathy

Parameter	Before study	3-months (Q_{10}+ALA+vit E)	Statistics
Plasma:			
CoQ_{10} (μmol/L)	0.572±0.050	1.204±0.090	p<0.0001
α-tocopherol (μmol/L)	23.94±2.10	30.91±0.24	p<0.0003
MDA (μmol/L)	5.23±0.21	4.51±0.19	p<0.0086
Echocardiographic parameters:			
EF /(%)	56.74±2.05	59.21±1.88	p<0.0004
LVEDD (mm)	53.21±0.87	54.90±0.81	p<0.0001
LVESD (mm)	39.74±1.01	38.47±1.07	p<0.0001
IVS (mm)	12.55±0.30	12.00±0.26	p<0.0001
PW (mm)	11.66±0.29	11.16±0.28	p<0.0001
E/A (ms)	0.66±0.01	0.67±0.02	p<0.0007
DT (ms)	291.80±5.42	284.74±5.36	p<0.0001
HbA_1c (%)	8.778±0.440	8.133±0.414	p<0.0005

Chronic supplementation of combined CoQ_{10}, ALA and vitamin E in patients with DCMP statistically significantly decreased HbA_1c values and oxidative stress; this treatment also had an antioxidant protective effect, improved echocardiographic parameters and myocardium function, without side effects. On the basis of this study CoQ_{10} and ALA with vitamin E treatment could be recommended in patients with DCMP as supplementation therapy [18, 53, 54].

Experimental Medicine

Diabetic mitochondrial cardiomyopathy and CoQ_{10}

The mitochondrion plays a central role in linking metabolism to insulin secretion from the pancreatic β-cells and this way blood glucose is regulated. Glucose enters the pancreatic β-cells and via cytosolic glycolysis is metabolised to pyruvate, which enters the mitochondrial citric acid cycle. Several mitochondrial mechanisms participate in diabetes [55]. The IMM is one of the major sites of ROS production and has a high content of PUFA. ROS generated in the mitochondrial respiratory chain may react with PUFA causing lipid peroxidation, alteration in the mitochondrial membrane integrity, irreversible swelling and disruption of mitochondria. Hyperglycemia induces oxidative stress due to increased mitochondrial superoxide anion production, nonenzymatic glycation of proteins and glucose autooxidation. Increased superoxide anion production by hyperglycemia could damage mitochondria and depletion mtDNA [56]. Impaired electron transport in Q-cycle can increase superoxide anion production in mitochondria in diabetes [57]. Decreased CoQ_9 and CoQ_{10} found in heart and liver mitochondria of rats with experimental diabetes mellitus participate in mitochondrial dysfunction [58]. High glucose

concentration leads to an increased reduction of equivalents such as NADH and $FADH_2$ within mitochondria. Their uptake from the cytoplasm occurs by various mitochondrial redox shuttles as well as by increasing uptake of pyruvate, which participates in ATP production from ADP and inorganic phosphate. The increased cytosolic ATP/ADP ratio causes closure of the plasma membrane K_{ATP} channels and depolarizes the β-cell. After depolarization of the plasma membrane, calcium influx into β-cells leads to secretion of insulin [59]. Mitochondrial dysfunction results in impaired glucose-stimulated insulin secretion [60].

The etiopathogenic mechanisms in chronic diabetic complications also include increased amount of contact sites in mitochondria [61]. Mitochondrial dysfunction has been documented in diabetes-associated cardiac complications [62]. Impairment of heart mitochondrial energy production gradually developed between weeks 4 and 21 weeks in rats with neonatally induced diabetes [63]. The acute phase of diabetes (8 days), adaptation of heart to diabetes was manifested by an increase in CoQ_{10} in heart mitochondria, increase in mitochondrial membrane fluidity, as well as by stabilization of membrane potential. These changes were associated with damaged mitochondrial respiration and ATP production [64].

Negative association between membrane fluidity and transmembrane potential offers new insight into acute diabetes-induced changes in cardiac mitochondria [65]. Mitochondrial permeability transition pore (MPTP), occurring with mitochondrial calcium overload and increased oxidative stress, seems to be another important factor which can lead to cardiac mitochondrial dysfunction in diabetes]66]. Cardiac pathological function undergoes circadian or circasemidian variations in the heart mitochondrial CoQ_{10} and oxidative phosphorylation, which may be a clue to the pathogenesis of the diabetic heart [16, 67].

In this experimental study diabetes was induced by a single *i.v.* injection of streptozotocin (55 mg/kg body weight). Three-month-old Wistar rats were synchronized during 60 days to 12 hours of darkness alternating with 12 hours of light, using Halberg's antiphasic lighting regimens. One L12:12 regimen was shifted to sample in the dark during waking hours.

Circadian variations of OXPHOS in mitochondria of diabetic rat heart

Circadian variations of OXPHOS in mitochondria of diabetic rats' heart show different time parameters at Complex I and Complex II, with two circadian maxima (at 24:00 and 04:00) and minima at 20:00. Table 7.

Circadian and circasemidian cascade of OXPHOS of diabetic rats' heart

For *Complex I*: maximal OXPHOS activity (PEAK 1, circadian) was between 08:59 and 19:20 (lasting 10.19 hours) during rats' inactivity. PEAK 2, circasemidian, during rats activity was between 00:29 and 11:34 (lasting 11.05 hours). For *Complex II*: maximal OXPHOS activity (PEAK 1) was between 03:25 and 11:31 (lasting 8.04 hours) during rats' inactivity and PEAK 2 was between 01:02 and 11:27 (lasting 10.25 hours) during rats' activity, - Table 8.

Comparison of circadian and circasemidian parameters of OXPHOS between control and diabetic rats' heart

Different circadian and circasemidian rhythms were found for OXPHOS between control and diabetic rats' heart. A statistically significant circadian cascade for parameters of OXPHOS (PEAK 1) was found in control hearts and circasemidian cascade (PEAK 2) in diabetic rats, Table 9.

Comparison of heart mitochondrial „Q_{10}-CLOCK" between control and diabetic rats

Nocturnal activity of rats (22:00-10:00) is reflected in higher peaks vs. diurnal peaks (10:00-22:00). This pilot study found statistically significant rat heart mitochondrial rhythm for CoQ_{10} circadians and circasemidians. PEAKS of CoQ_{10} were at 15:27 and 03:30. NADIRS of CoQ_{10} were at 10:00 and 20:51 in control hearts. Different PEAKS and NADIRS were found in diabetic rats' heart: PEAKS were at 13:09 and 02:18 and NADIRS were at 08:58 and 19:49, Figure 7.

Table 7. Circadian variations of OXPHOS in mitochondria of diabetic rat's heart

Hours	8:00	12:00	16:00	20:00	24:00:00	4:00
Complex I.						
ADP:O (nmol/nAtO)	2.424	2.782	3.068	2.860	3.029	3.133
OPR (nmol ATP/mg prot.min)	293.31	309.30	322.73	**202.72**	**425.88**	**261.40**
S3 (nAtO/mg prot/min)	158.00	144.85	135.89	**95.51**	**186.60**	**104.90**
S4 (nAtO/mg prot/min)	54.95	33.78	26.50	31.84	34.56	27.13
RCI (S3/S4)	2.875	4.295	5.165	3.000	5.400	3.875
Complex II.						
ADP:O (nmol/nAtO)	1.690	1.856	1.722	2.015	1.566	1.365
OPR (nmol ATP/mg prot.min)	376.76	227.75	247.08	**135.38**	**384.84**	**430.30**
S3 (nAtO/mg prot/min)	253.12	185.00	190.42	**86.73**	**338.45**	**341.31**
S4 (nAtO/mg prot/min)	149.52	124.19	116.37	56.37	195.26	211.43
RCI (S3/S4)	1.695	1.490	1.640	1.540	1.730	1.610

Table 8. Circasemidian and circadian parameters of cascade of OXPHOS in diabetic rat's heart

	PEAK1 (24 h)		PEAK 2 (12 h)	
	(parameter)	(hour)	(parameter)	(hour)
Complex I	**S4**	8:59*	**S4**	9:34
	S3	11:26	**S3**	11:34
	RCI	14:18*	**OPR**	0:29
	ADP:O	15:42*	**RCI**	1:31
	OPR	19:20	**ADP:O**	2:18
Complex II				
	RCI	3:25	**ADP:O**	8:18
	ADP:O	8:44*	**OPR**	11:27
	OPR	9:16	**S4**	1:02
	S3	10:40	**S3**	1:12
	S4	11:31*	**RCI**	8:12

* statistically significant

Table 9. Comparison of circadian and circasemidian parameters of OXPHOS between control and diabetic rats' heart mitochondria

		PEAK 1 Control (h)	PEAK 1 Diabetes (h)	NADIR 1 Control (h)	NADIR 1 Diabetes (h)	PEAK 2 Control (h)	PEAK 2 Diabetes (h)	NADIR 2 Control (h)	NADIR 2 Diabetes (h)
	parameter								
Complex I	**S4**	9:02*	8:59*	23:42	15:42	23:42	21:34*	4:18	2:18
	ADP:O	9:16*	15:42*	2 4 : 00	9:27	2 4 : 00	2:18*		21:05
	OPR	10:47*	19:20	0:58	18:07	0:58*	0:29*		7:24
	S3	11:02*	11:16	1:13	17:16	1:13	23:34*	6:07	5:09
	RCI	14:22*	14:18*	2:14	20:33	2:14*	8:00*		8:00
Complex II	**S4**	10:04*	11:31*	4:33	18:14	4:33	1:02		
	ADP:O	10:04*	8:44*	0:11	14:00	0:11*	20:18*	5:10	2:39
	OPR	10:14*	9:16	1:02	16:29	1:02	23:27		4:07
	S3	12:18*	10:40	3:02	17:38	3:02	1:12		
	RCI	12:51	3:25	6:34	14:22	6:34	8:12	7:38	

PEAK1, NADIR 1 - circadian (24 hours)
PEAK 2, NADIR 2 - circasemidian (12 hours)
hours (h)
* statistically significant

***Conclusions*:** Mitochondrial „Q_{10}-CLOCK“ appears as a key parameter for regeneration of mitochondrial membrane and re-energization of heart mitochondria of control and diabetic rats. Mapping changes in heart mitochondrial CoQ_{10} and ATP production along the 24-hour scale can contribute a better understanding of the triggering of acute heart attacks [14].

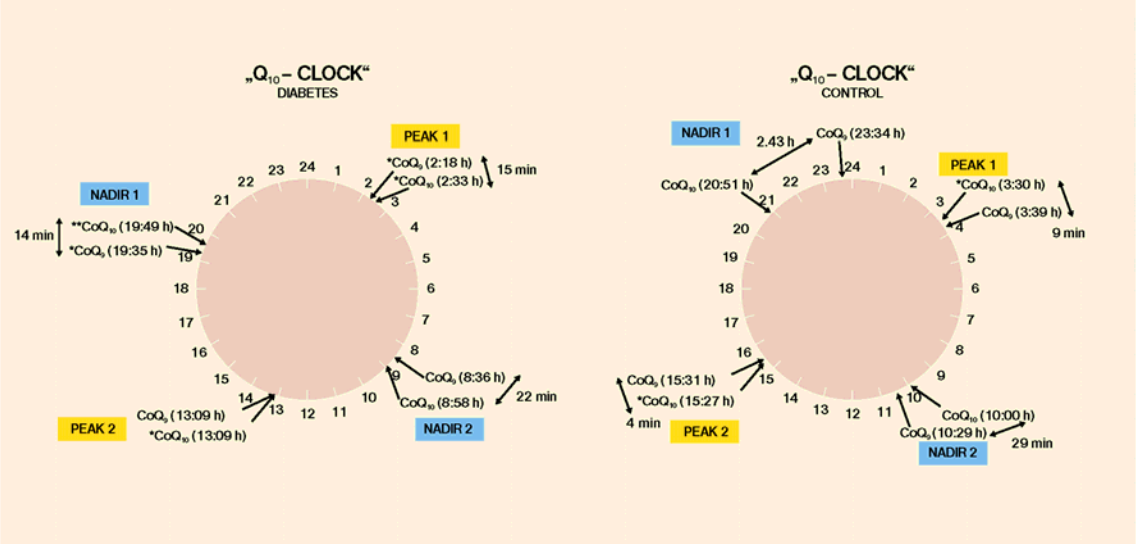


Figure 7. Comparison of heart mitochondrial „Q_{10}-CLOCK“ between control and diabetic rats.

Conclusion and Perspectives

Mitochondrial dysfunction has been associated with cardiomyopathy of unknown etiology, and it has been involved in the pathobiochemical mechanisms of rejection of human transplanted heart and diabetic cardiomyopathy. Circadian and ultradian changes in heart mitochondrial cascades of OXPHOS and „CoQ_{10}-CLOCK“may play an important role in mitochondrial cardiomyopathies. Mapping changes in heart mitochondrial „CoQ_{10}-CLOCK“ and ATP production along the 24-hour scale can contribute a better understanding of the triggering of an acute heart attack. Damaged mitochondria could be a target for drugs and antioxidants therapy (including CoQ_{10}), providing their regeneration in various human diseases.

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Circadian Cardiomyocyte Function and Cardiomycyte Circadian Clock

NS Verma[*1], RK Singh[2], RB Singh[3], Jan Fedacko[4], Krasimira Hristova[5], Anna Gvozdjáková[6], Branislav Milovanovic[7], Toru Takahashi[8], DW Wilson[9], and NS Dhalla[10]

[1]Department of Physiology, KG Medical University, Lucknow, India
[2]Department of Biochemistry, KG Medical University, Lucknow, India
[3]Halberg Hospital and Research Institute, Moradabad, India
[4]PJ Safaric University, Kosice, Slovakia
[5]National Heart Hospital, Sofia, Bulgaria
[6]Pharmacobiochemical Laboratory of 3rd Department of Internal Medicine, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic
[7]Department of Internal Medicine and Cardiology, University Clinical Hospital, Belgrade, Serbia
[8]Graduate School of Environmental Sciences, Fukuoka Women's University, Fukuoka, Japan
[9]School of Medicine, Pharmacy and Health, Durham University, Durham, UK
[10]Institute of Cardiovascular Sciences, Winnipeg, Canada

Abstract

Background. Cardiomyocyte circadian rhythms and the circadian clock are known to coordinate myocardial function. In this review we discuss whether disruption of this mechanism plays a potential role in the etiology of cardiovascular diseases (CVDs), obesity and type 2 diabetes.

Methods. Internet search and discussion with colleagues.

Results. Circadian clock genes have been identified and characterized within almost all mammalian cell types, including cardiomyocytes, vascular smooth muscle cells, endothelial cells, and fibroblasts. Clocks are found in both prokaryotes and eukaryotes. However, a few reports suggest that specific prokaryotes may not possess functional clocks. Circadian clocks are transcriptionally-based cell autonomous molecular mechanisms that directly coordinate cellular/biological functions at multiple temporal levels. As reported by various workers, a number of roles played by circadian clock genes within the cardiovascular system are composed of both positive and negative feedback loops, with a free-running period of approximately 24 hours. The cardiomyocyte circadian clock influences myocardial contractile function, metabolism, and gene expression and coordinates myocardial oxygen consumption and fatty acid oxidation rates. Experimental mice constitute a model of temporal suspension of the heart circadian clock at the wake-to-sleep transition, which is distinct from classic models of shift work involving manipulation of the light-dark cycle. The expression of approximately 10-15% of all myocardial genes oscillate in a time-of-day-dependent manner which is clear from hearts collected from mice under light/dark (24-hour synchronized) and constant dim light (free-running) conditions. The major fuel sources for continued contraction of the myocardium are fatty acids and glucose, which may be an integral component of the cardiomyocyte circadian clock. In the oxidative myocardial metabolism, circadian variations in the rat's heart revealed time-of-day-dependent oscillations in glucose, but not in fatty acid, oxidation. There is a greater transcriptional response when the rat's heart is challenged with fatty acids during the active phase. This response is mediated by the cardiomyocyte circadian clock, and it is potentially mediated by clock-controlled oscillations in nuclear receptors, such as PPARα, Rev-erbα, and PGC1α. The circadian system is a complex feedback

[*] **Correspondence:** Dr N S Verma, MD. Professor, Department of Physiology, K G Medical University, Lucknow (UP) 226003, India, narsinghverma@gmail.com

network that involves interactions between the central nervous system and peripheral tissues as well as the metabolic system. It is possible that circadian coordination is intimately linked to metabolic homeostasis and that dysregulation of circadian rhythms can contribute to cardiometabolic risk. It seems that metabolic signals also feed back into the circadian system, modulating circadian gene expression and altering their behavior, resulting in an increased risk of CVDs, obesity, and diabetes.
Conclusions. Recent molecular- and genetic-based studies suggest that the cardiomyocyte circadian clock influences multiple myocardial processes, including transcription, signaling, growth, metabolism, and contractile function. In view of its physiological roles, the cardiomyocyte circadian clock has recently been linked to the pathogenesis of heart disease in response to adverse stresses, such as ischemia /reperfusion, in animal models as well as to the risk of obesity and type 2 diabetes.

Keywords. Heart, myocardial, circadian, metabolic function

Introduction

Circadian timing presents a selective advantage at multiple biological tiers, including cellular, tissue, and whole organism levels [1-3]. Intracellular molecular mechanisms that allow the cell to anticipate the time of day may be defined as circadian clocks [2]. This information is critical to ensure appropriate and rapid responses which are achieved in a temporally appropriate manner. The ability to prepare for an event before it occurs is critical for numerous aspects of life, possibly at the cellular level, the binding of a hormone to its receptor to ensure an appropriate cellular response. The intact rat's heart expresses the major components of the circadian clock, of which its rhythmic expression in vivo is consistent with the operation of a fully functional clock mechanism. The differences between pathological and physiological cardiac hypertrophy lead to novel therapeutic strategies to treat heart failure depending on circadian rhythms [4]. The role of the time structure on biochemical and biological function was pointed out by Franz Halberg long before Bartter [5-7]. Halberg went on conducting research starting from molecular medicine to clinical cardiology which is popularly known as chronocardiology, the name given by him [7].

Interestingly, an experimental study exposed the oscillations of circadian clock genes [brain and arylhydrocarbon receptor nuclear translocator-like protein 1 (*Bmal1*), reverse strand of the *c-erbaα* gene (*rev-erbaα*), period 2 (*per2*), albumin D-element binding protein (*dbp*)] for isolated adult rat's cardiomyocytes in culture [1]. The findings suggest that the circadian clock operates within the myocytes of the heart. It is interesting that this molecular mechanism persists under standard cell culture con ditions (i.e., 2.5% serum). Norepinephrine, unlike glucose, influences the timing of the circadian clock within the heart, and the circadian clock may be a novel mechanism coordinating myocardial metabolism. The purpose of this article is to highlight our current knowledge regarding the functions of the cardiomyocyte circadian rhythm and circadian clock, and to discuss whether disruption of this mechanism plays a potential role in the etiology of cardiovascular disease (CVDs), obesity and type 2 diabetes. Since several reviews have been published recently on this topic, this article will rapidly focus on distinct areas of novel ongoing research.

Circadian Rhythm in Myocardial Function

The myocardial responsiveness to alterations in pressure/sheer stress occurs on a daily basis within a physiological range, peaking during the active/awake span, a time when physical activity is typically elevated [2, 3]. Increased physical activity may promote adaptation in a physiological hypertrophy of the myocardium. However, a persistent elevation of sheer stress results in a pathological hypertrophic response [4]. It is possible that an inappropriate elevation of sheer stress during the inactive/sleep phase, which is observed among non-dipping hypertensives, may contribute to a pathological response resulting in left ventricular dysfunction. However, an appropriate anticipation of temporal changes in sheer stress may result in a physiological hypertrophy of the heart.

Dipping of blood pressure at night compared to daytime is a circadian response of our body. This response is physiological and protective against environmental factors.

The main criterion for biological anticipation of a cell is a stimulus or trigger at a distinct time of the

day, which may be circaseptan or ultradian as well as a molecular time keeping mechanism with reasonable plasticity synchronized by the environment.

The molecular adjustment of our body has evolved such a time keeping mechanism which is the circadian clock. Regular circadian physiological variation in eosinophil counts in five stocks of mice has been observed and reported by Halberg for the first time in the literature in 1950 [5]. The words circadian and chronobiology were used for the first time by Franz Halberg in 1950 and officially introduced to a nomenclature committee in Stockholm in 1955 [5, 6]. Chonobiology developed globally, after1969, when an article entitled "Chronobiology" was published in the Annual Review of Physiology and became a Current Contents Citation Classic [5-7].

The Heart Circadian Clock

Circadian clocks have been identified and characterized within almost all mammalian cell types, including cardiomyocytes, vascular smooth muscle cells, endothelial cells, and fibroblasts [1, 8, 9]. Clocks are found in both prokaryotes and eukaryotes. However, a few reports suggest that specific prokaryotes may not possess functional clocks [8]. Circadian clocks are transcriptionally-based cell autonomous molecular mechanisms that directly coordinate cellular/biological function at multiple temporal levels [1, 8]. One study reported thrombo modulin to be a clock-controlled gene in vascular endothelial cells [9]. A number of roles of circadian clocks within the cardiovascular system have been reported by various investigators. New developments include observations that mouse models of circadian clock dysfunction exhibit alterations in endothelial function and blood pressure as well as variations in vascular injury susceptibility [10, 11]. There may be an increased risk of hypertension in humans due to distinct genetic polymorphisms in circadian clock gene components (e.g., Bmal1) [12].

Circadian clocks may be characterized as a transcriptionally-based molecular mechanism, composed of both positive and negative feedback loops, with a free-running period of approximately 24 hours [1-3]. In view of the autonomous nature of this mechanism of the cell, circadian clock component gene expression oscillations observed in the intact heart persist in isolated cultured myocardial tissue and cardiomyocytes [1, 13-15]. It has been observed that clock genes display rhythmic expression in human hearts [15].Time structured oscillations in clock component gene expression have been characterized in both rodent and human hearts [13-15]. Since this mechanism is transcriptional, several laboratories have investigated oscillations in the transcriptome of the heart over the course of the day [16]. The reported oscillations in gene expression in normal intact hearts could be mediated by neurohumoral as well as cardiomyocyte circadian clock influences.

Apart from identification of clock-controlled genes (CCGs), phenotypic characterization of cardiomyocyte-specific clock mutant (CCM) mice has been useful for revealing novel roles of the heart circadian clock on myocardial function. In this mouse model, the contribution of the cardiomyocyte circadian clock mechanism was selectively disrupted [17]. The CCM mouse is a model of temporal suspension of the heart circadian clock at the wake-to-sleep transition which is distinct from classic models of shift work involving manipulation of the light-dark cycle [18]. The expression of approximately 10-15% of all myocardial genes oscillate in a time-of-day-dependent manner which is clear from hearts collected from mice under light/dark (24-hour synchronized) and constant dim light (free-running) conditions [19, 20]. In clinical studies, night shift work is known to predispose for CVDs [21, 22]. A recent study compared 14 healthy nursing professionals aged 20-40 years performing day and night shifts with 14 control subjects performing day duty. Circadian patterns of blood pressure and heart rate were evaluated in night shift workers during the work shift (night and day shift) and in controls [22]. Night shift workers showed a very interesting altered circadian amplitude when they went back to the day shift. Statistically significantly differences in the circadian amplitude of SBP and DBP were found between day shift workers and controls ($P<0.01$). Night shift work or late sleep may be associated with increased release of cortisol and ghrelin and decreased release of melatonin and leptin, which have adverse effects on cardiometabolic risk factors, leading to insulin resistance [21, 22]. Disruption of the circadian clock within the cardiomyocyte influences myocardial

contractile function, metabolism, and gene expression [23, 24].

The role of the intrinsic heart circadian clock in the transcriptionally based molecular mechanism in cardiovascular biology is poorly understood [23]. It is possible that the circadian clock within the cardiomyocyte influences circadian variations in myocardial biology. In an experimental study, CCM mice exhibit normal myocardial contractile function in vivo, as assessed by echocardiography [24]. There was an attenuation of heart rate circadian variations and bradycardia in CCM mice (in the absence of conduction system abnormalities), as revealed by radiotelemetry studies. The intrinsic nature of this phenotype was indicated by reduced heart rate that persisted in CCM hearts perfused ex vivo in the working mode. Gene expression microarray analysis identified 548 and 176 genes in atria and ventricles, respectively. The usual circadian expression patterns of the genes were altered in CCM mice. These studies suggest that the cardiomyocyte circadian clock influences myocardial contractile function, metabolism, and gene expression. The myocardial oxygen consumption and fatty acid oxidation rates were increased. Cardiac efficiency was decreased in CCM hearts, but there were no alterations in mitochondrial content or structure and modest mitochondrial dysfunction in CCM hearts.

Myocardial Metabolism

The contractile function of the myocardium is related to myocardial metabolism and any impairment of energy metabolism can have adverse effects on cardiac function [25, 26]. There are increased metabolic fluxes to meet energetic demands during conditions of increased cardiac output such as during physical activity or mental stress [27]. In several feedback loops of the mammalian circadian clock, there are integrated metabolic functions.

The major fuel sources for continued contraction of the myocardium are fatty acids and glucose, which may be an integral component of the cardiomyocyte circadian clock [25]. In the oxidative myocardial metabolism, circadian variations in the rat's heart revealed time-of-day-dependent oscillations in glucose, but not in fatty acid, oxidation. It seems that under reference non-stressed conditions, the cardiomyocyte circadian clock does not directly influence myocardial oxidative metabolism. However, in isolated adult rat's cardiomyocytes, neither glucose nor fatty acids acutely influences the expression of circadian clock components [1, 17].

Coordination of the responsiveness of the heart to fatty acids and a metabolic link with the cardiomyocyte circadian clock seems interesting. Increase in fatty acid concentrations may increase both oxidative and non-oxidative fatty acid metabolism in the myocardium, in an attempt to maintain intracellular fatty acid concentrations within a physiological range. In the rat's heart, both the acute and chronic responsiveness of the myocardium has a time-of-day-dependence, challenging the heart with fatty acids during the sleep phase results in an acute depression of cardiac output and efficiency, a phenomenon that is not observed during the active phase [1, 17, 27, 28]. However, challenging the rat's heart with fatty acids during the active phase results in a greater transcriptional response at this time which is mediated by the cardiomyocyte circadian clock, and it is potentially mediated by clock controlled oscillations in nuclear receptors, such as PPARα, Rev-erbα, and PGC1α [27-32]. It is possible that the temporal-dependence, such as consumption of dietary lipid at one time of day compared to other times has profound effects on the cardiovascular system as well as on whole body energy homeostasis, which may influence susceptibility to weight gain, adiposity, insulin resistance and CVDs.

Increased protein synthesis required for hypertrophy also involves several translation initiation factors which may be important in protein synthesis coordination because the circadian clock is known to influence protein degradation. The protein ubiquitination, degradation and turnover appear to be integral processes in the circadian clock mechanism for the removal of damaged proteins from the cell [33]. Increased protein turnover is also important during both stages of myocardial growth and atrophy [34-36]. The point is that the cardiomyocyte circadian clock coordinates multiple components of the ubiquitin/proteosome system (e.g., *usp2*, *ubc*, *ube3c*) [24]. Further studies on protein turnover rates in WT and CCM hearts are likely to provide important

insight into cardiomyocyte circadian clock coordination of myocardial growth.

Thrifty Genes and Circadian Clock and Development of Heart Disease and Diabetes

The temporal organization of many aspects of physiology, including metabolism, in synchrony with the 24-hour rotation of the Earth may be under the influence of thrifty genes [37-39]. The circadian system is a complex feedback network that involves interactions between the central nervous system and peripheral tissues as well as the metabolic system. It is possible that circadian coordination is intimately linked to metabolic homeostasis and that dysregulation of circadian rhythms can contribute to cardiometabolic risk. It seems that metabolic signals also feed back into the circadian system, modulating circadian gene expression and altering the behavior resulting in the increased risk of CVDs, obesity, and diabetes. In 1962, Neel proposed the thrifty gene hypothesis, indicating that humans evolved to store excessive calories as triglyceride in adipose tissues, during times of ample nutrient supply in summer, in anticipation of prolonged stages of minimal nutrient availability during winter [37]. The current lack of seasonal changes in nutrient supply in the industrialized world in association with this strong genetic susceptibility, potentially contributes to our ongoing obesity epidemic [37-39]. Important candidate thrifty genes have been identified through numerous human genomic approaches, and include leptin, MCR4, and POMC [38, 39].

In the majority of the CVDs (hypertension, various cardiomyopathies, myocardial infarction and conduction disorders), a new generation of atrial and/or ventricular fibrosis has been implicated [40, 41]. Fibrosis in the heart has been associated with an increase in atrial and ventricular tacchyarrythmia and sudden cardiac death, both of which exhibit a strong circadian rhythm in patients [42, 43]. Fibrosis in the atria predisposes to atrial fibrillation [44]. Similarly, increased fibrosis has been linked to decoupling of muscle fibers, conduction slowing, and conduction blocks [45, 46]. Degradation of the existing extracellular matrix (ECM) by extracellular matrix metalloproteinases (MMPs) initiates inflammation, resulting into fibrosis. Chronic heart failure, myocardial infarction, and hypertension, have also been reported to be associated with increased concentrations and activities of MMPs as well as fibrosis [47-49]. Coenzyme Q10, ω-3 fatty acids and antioxidant enzymes present in the cell membrane can inhibit the activity of MMPs, which is also normally counterbalanced by the presence of tissue inhibitors of matrix metalloproteinases (TIMPs) [49]. MMP concentrations have been shown to be elevated and TIMP concentrations repressed during times of ECM breakdown, which is followed by a decrease in MMP activity (by TIMP inhibition) and collagen deposition [50]. There is an enrichment of genes involved in the turnover of extracellular matrix and deposition of collagen fibers as being coordinated by the cardiomyocyte circadian clock.

Recent studies indicate that circadian misalignment could be important in the development of obesity, diabetes mellitus, and CVDs [24]. Time-of-day-dependent synchronization of organisms with their environment is mediated by circadian clocks. This cell autonomous mechanism has been identified within all cardiovascular-relevant cell types, including cardiomyocytes. Recent molecular- and genetic-based studies suggest that the cardiomyocyte circadian clock influences multiple myocardial processes, including transcription, signaling, growth, metabolism, and contractile function. In view of its physiological roles, the cardiomyocyte circadian clock has recently been linked to the pathogenesis of heart disease in response to adverse stresses, such as ischemia/reperfusion, in animal models [43-50].

The mechanism of the circadian clock evolved to provide the selective advantage of anticipation in which the clock ensures that biological events occur at a temporally appropriate time of day [1-3]. One evolutionary pressure for circadian clock selection includes coordination of the dark phase, in which the circadian clock ensures that light phase induced DNA damage (i.e., UV irradiation) is not inadvertently transmitted to daughter cells [51]. Metabolic challenges imposed on the cell also have a strong and predictable time-of-day-dependence and metabolism has integrated into the circadian clock mechanism [52, 53] (Figures 1 and 2).

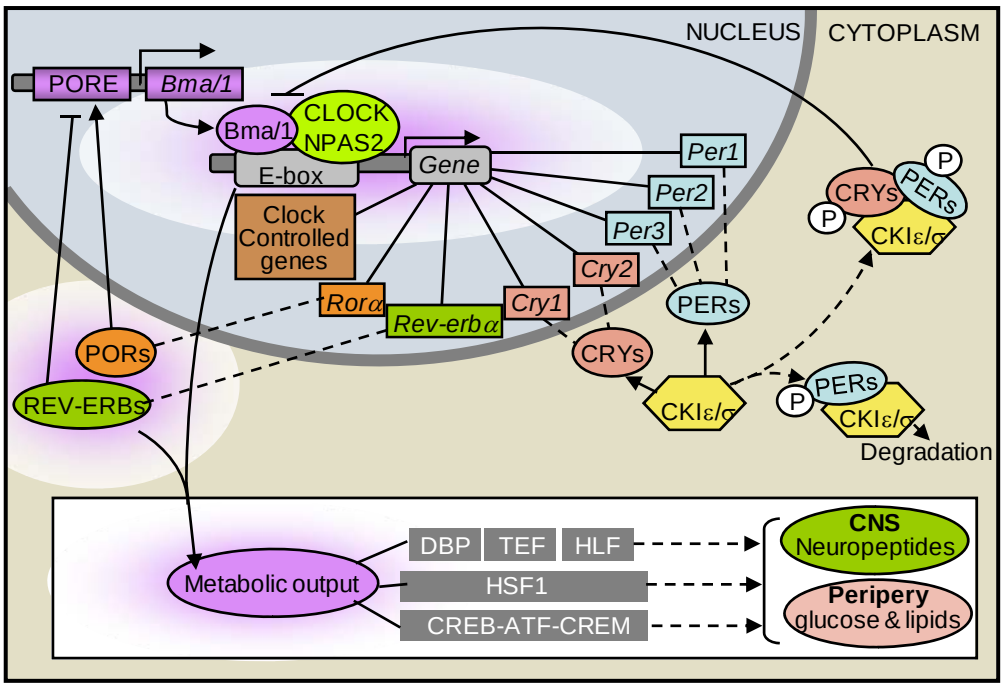

Figure 1. Showing interaction of genetics with clock encoded by a transcription-translation feedback loop that oscillates with a periodicity of 24 hr in pacemaker neurons and peripheral cells.

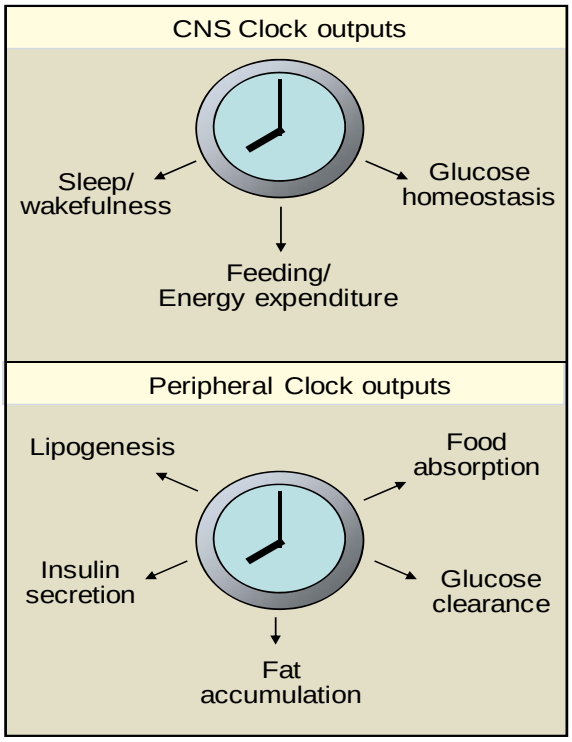

Figure 2. The master pacemaker encoding the mammalian clock resides within the suprachiasmatic nucleus (SCN), although clock genes are also expressed in other regions of the brain and in most peripheral tissues.

The cardiomyocyte circadian clock research indicates that this mechanism may allow the heart to anticipate prolongation of the sleep-phase fast, when the animal in the wild is initially unsuccessful in its forage for food [53]. The role of amino acids in relation to the cardiomyocyte function and dysfunction could be crucial because of their effects on nitric oxide release [54]. The data indicate that phoslipase isozymes may coordinate their own gene expression through a PKC and ERK 1/2-dependent pathway in a cycle of events which might be circadian in nature, and may be related to the cardiomyocyte hypertrophic response [55]. It may be evolutionarily advantageous to anticipate times of prolonged fasting, as it has been suggested previously, and it may account in part for the increased prevalence of cardiometabolic disease in Western society and in newly developed or rapidly developing populations.

In brief, the circadian cardiomyocyte clock coordinates myocardial function in circadian cycles, resulting in physiological benefit during daytime activity. However, at night, physical activity may have a pathological effect on myocardial function. It is possible that circadian coordination is intimately linked to metabolic homeostasis and that dysregulation of circadian rhythms can contribute to cardiometabolic risk leading to CVDs, obesity and type 2 diabetes.

The authors declare that there is no conflict of interest.

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Mathematical Model of Cardiovascular Disease Risk Based on Vascular Variability Disorders

J. Siegelova*[1], J. Dusek[1], K. Otsuka[2], and G. Cornelissen[3]

[1]Department of Physiotherapy, Department of Sports Medicine and Rehabilitation, Masaryk University, Brno Czech Republic

[2]Tokyo Women's Medical University, Medical Center East, Tokyo, Japan

[3]Halberg Chronobiology Center, University of Minnesota, Minneapolis, MN, USA

Abstract

Blood pressure (BP) values during ambulatory BP monitoring are mostly still interpreted conventionally in the light of the same fixed 24-hour, daytime and nighttime limits for all adults 18 years and older. We show that a chronobiologic approach taking into consideration the circadian characteristics of the BP waveform adjusted for gender and age enable a better estimation of cardiovascular morbidity and mortality risk. Apart from increased 24-hour mean values of BP (MESOR, short for Midline Estimating Statistic of Rhythm), an attribute of MESOR-hypertension (MH), altered patterns of variability in BP and heart rate (HR) are markers of cardiovascular disease risk. Screening for these Vascular Variability Disorders (VVDs) yields a refined diagnosis and prognosis regarding the risk of cardiovascular morbidity and mortality. It also serves as a guide for timed treatment (chronotherapy) when warranted. This chronobiologic approach based on ambulatory BP monitoring remains applicable to manual measurements taken at intervals from the time of awakening to bedtime, preferably with an added measurement around mid-sleep.

Key words: ambulatory blood pressure monitoring (ABPM), essential hypertension, Vascular Variability Disorders (VVDs), heart rate variability (HRV)

Introduction

From the viewpoint of lost years of healthy life, hypertension is the most important disease globally. Treatment of hypertension can avert adverse events such as stroke, myocardial infarction or cardiac death, while side effects are relatively mild. Conventional diagnosis of hypertension still relies on the more-than-century-old method of measuring BP in the doctor's office. The shortcomings of this approach are already known. The 1980 Australian study showed that 48% of the 1943 patients who entered the placebo arm of the trial were "cured" after 3 years [1]. The high BP variability and the regression to the mean statistical phenomenon may account for this situation.

* ***Correspondence****:* Prof. MUDr. Jarmila Siegelová, DrSc. Department of Physiotherapy. Department of Sports Medicine and Rehabilitation. Faculty of Medicine, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic. FN u sv. Anny v Brne, Pekarska 53, 656 91 Brno, Czech Republic. e-mail: jsiegel@med.muni.cz, jarmila.siegelova@fnusa.cz

Franz Halberg, the founder of chronobiology, recognized early on the importance of assessing the circadian rhythm in BP as a gauge of cardiovascular disease risk. In the experimental laboratory, an elevation of the circadian amplitude of BP preceded the increase in MESOR in the stroke-prone Okamoto rat [2]. First self-measurements, then ambulatory monitoring of BP in school children showed that a positive family history of high BP and/or related cardiovascular disease was associated with a larger circadian amplitude of BP [4], a finding later also found in neonates [5]. We published the first Czech study using ABPM 20 years ago together with Professor Bohumil Fiser [5]. Nevertheless, modern guidelines for the diagnosis and treatment of hypertension [6] do not give priority to ABPM as a first-choice method, even though the financial cost of ABPM has markedly decreased.

Home BP measurements several times a day can also be carried out by the patient using low-cost automatic devices, albeit taking a measurement around mid-sleep is important to obtain reliable estimates of the circadian characteristics [7]. The higher reliability of diagnosis achieved by around-the-clock sampling of BP interpreted chronobiologically helps refine the treatment plan and increase patients' compliance with the treatment regimen, making it also possible to focus on patients at a higher risk of target organ damage, leading to adverse events and premature death. Screening for Vascular Variability Disorders (VVDs) by identifying deviations from norms in BP and/or HR variability should be done on the basis of long-term ABPM or home measurements of BP [8].

Vascular Variability Disorders

As outlined earlier [9, 16], VVDs established on the basis of ABPM include:

1. MESOR-hypertension, an elevation of the systolic (S) and/or diastolic (D) BP MESOR above the upper 95% prediction limit of clinically healthy peers matched by gender and age. The MESOR is a rhythm-adjusted mean obtained by fitting a single- or multiple-component model to the data by cosinor. A 2-component model consisting of cosine curves with periods of 24 and 12 hours is usually used for screening for VVDs. The MESOR is usually more precise and more accurate than the arithmetic mean.
2. CHAT (Circadian Hyper-Amplitude-Tension), an elevation of the circadian amplitude of SBP and/or DBP MESOR above the upper 95% prediction limit of clinically healthy peers matched by gender and age. Outcome studies have shown that this condition of BP over-swinging is associated with an increased risk of morbidity and mortality in patients due to cardiovascular diseases like coronary artery disease, stroke, and renal failure. CHAT's definition is based on the fit of a cosine model to the data. The model accounts for the fact that BP is usually lower at night during the rest span than during the active daytime. As the difference between daytime and nighttime BP values is approximately twice the circadian amplitude estimated by cosinor, it has been suggested to use the day-night ratio as an approximation of the circadian variation for a classification in terms of dipping [17]. Risk assessment has been more reliable, however, when it is based on the circadian amplitude and acrophase than on the day-night ratio [18-21].
3. An excessive brachial pulse pressure (difference between the SBP and DBP MESORs above 60 mmHg).
4. Deficient HRV, defined in terms of the standard deviation (SD) of HR below 7.5 beats/min.
5. An odd circadian timing of the BP but not of the HR rhythm. This pattern is sometimes observed in patients with type 2 diabetes complicated by autonomic nervous dysfunction [22-24].
6. Deviation of the circadian period from 24 hours. This pattern has been observed in a woman suffering from recurring adynamic depression, as discussed elsewhere [25].
7. An excessive pulse pressure product (SBP-MESOR.HR-MESOR/100>100 mmHg.beats/min.%) [16].

Risk Assessment

While further risk of cardiovascular disease increases linearly with increased values of the MESOR of SBP and DBP, the risk associated with an excessive circadian amplitude of BP or a decreased HR-SD only appears after a threshold value has been crossed [26]. This can be seen in Figure 1 showing the relationship between the risk for at least one of the events (coronary artery disease, stroke, nephropathy and retinopathy) within 6 years of the 48-hour ABPM and the MESOR or the (double) amplitude of SBP and DBP.

Table 1 shows the magnitude of the risk for various VVDs present alone or in combination with other VVDs. The results stem from a study on 297 patients [9, 10]. They were confirmed in several other studies, including many more patients [11, 14, 16, 19-21, 26].

Table 1. Risk of any adverse event (coronary artery disease, cerebral ischemic event, retinopathy, nephropathy) within 6 years of ABPM (expressed as percentage incidence per group)

Primary VVD:	Additional VVDs			
	0	1	2	3
MH	9%	29%	53%	100%
CHAT	29%	13%	44%	100%
EPP	--	34%	54%	100%
DHRV	0%	40%	62%	100%

VVD: Vascular Variability Disorder; MH: MESOR-Hypertension; CHAT: Circadian Hyper-Amplitude-Tension; EPP: Excessive Pulse Pressure; DHRV: Deficient Heart Rate Variability.

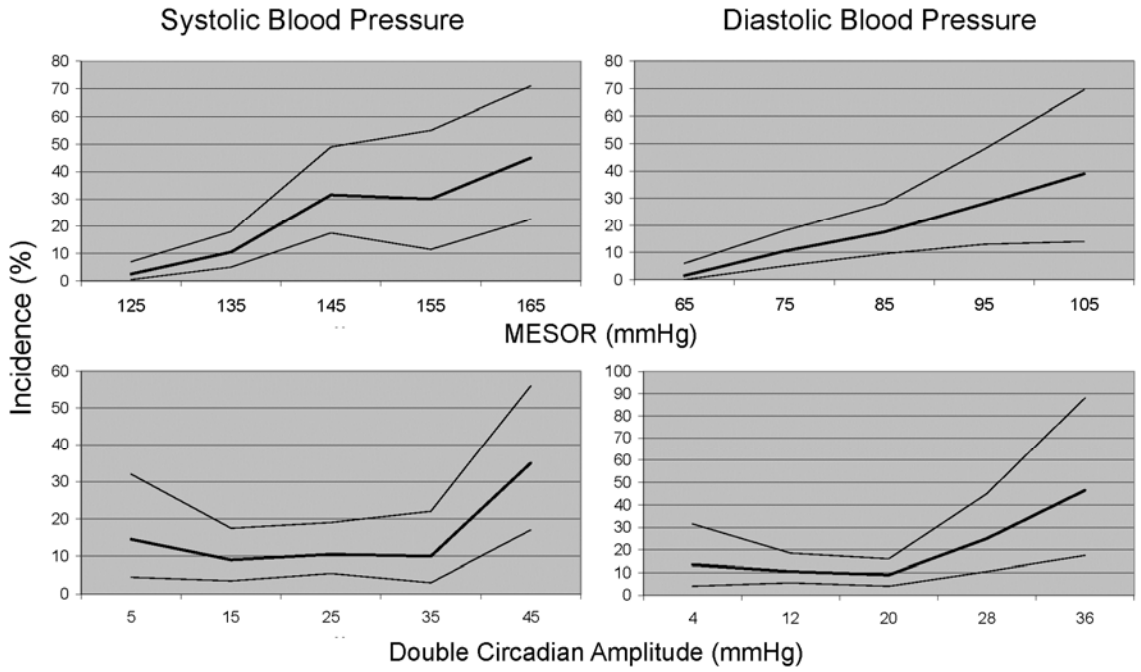

Figure 1. Relationship between the risk of any adverse event (coronary artery disease, cerebral ischemic event, retinopathy, nephropathy) within 6 years of ABPM (expressed as percentage incidence per group, shown on the ordinate) and the MESOR (top) or double circadian amplitude (bottom) of SBP (left) and DBP (right). Thin lines correspond to 95% confidence limits.

Sampling Requirements

The basis for a reliable diagnosis of MESOR-hypertension and other VVDs is to obtain sufficient BP measurements. Calculations can be performed on the basis of a single 24-hour record, but in view of the large day-to-day variability in circadian characteristics seen in many patients [20, 27-29], it has been recommended to monitor for at least 7 days at the outset, with measurements taken every 30-minute intervals [15, 16]. It is of course possible to combine data from ABPM with home BP measurements. When doing so, it is necessary to obtain several BP values also during the rest span, that may be taken during sleep by some family member [7]. If the patient wakes up at night, then it is very appropriate to take a measurement in bed before getting up.

Mathematical approach for Identifying VVDs and Assessing Cardiovascular Disease Risk

The calculation can be performed for the series as a whole and also for each day separately. The model fitted to the data is

$$Y(t) = M + A_1.\cos(2\pi t/\tau_1 + \phi_1) + A_2.\cos(2\pi t/\tau_2 + \phi_2) + e(t).$$

Since the periods τ_1 and τ_2 are fixed (24 and 12 hours, respectively), the equation can be rewritten as

$$Y(t) = M + \beta_1 x_1 + \gamma_1 z_1 + \beta_2 x_2 + \gamma_2 z_2 + e(t)$$

where

$$\beta_1 = A_1\cos(\phi_1);\ \gamma_1 = -A_1\sin(\phi_1);\ \beta_2 = A_2\cos(\phi_2);\ \gamma_2 = -A_2\sin(\phi_2);$$
$$x_1 = \cos(2\pi t/\tau_1);\ z_1 = \sin(2\pi t/\tau_1);\ x_2 = \cos(2\pi t/\tau_2);\ z_2 = \sin(2\pi t/\tau_2).$$

The model is now linear in its parameters (M, $\beta_1, \gamma_1, \beta_2, \gamma_2$). Obtaining estimates for these parameters can be done in Excel using the linear regression function, where the independent variables are x_1, z_1, x_2, and z_2. Estimates for A_1, ϕ_1, A_2, and ϕ_2 are then obtained using the following relations

$$A = (\beta^2 + \gamma^2)^{1/2}$$

$$\phi = \arctan(-\gamma/\beta) + K\pi \text{ where K is an integer.}$$

Reference values for M, A_1 and ϕ_1 are calculated as 90% prediction limits from records of clinically healthy subjects, separately for men and women in different age groups [30]. Deviation of the period from 24 hours needs to be assessed by nonlinear least squares [31, 32]. Until gender- and age-specific reference values are derived for the pulse pressure, HR-SD, and the pulse pressure product, threshold values of 60 mmHg, 7.5 beats/min, and 100 mmHg.beats/min.% have been used, respectively.

Discussion

The diagnosis of MESOR-hypertension is similar to the classic definition of hypertension based on the mean values of SBP and DBP from the ABPM record. The major differences relate to its calculation which is more robust when some data are missing or taken at irregular intervals, and to the fact that reference values are gender- and age-specific. Essential hypertension is known as a risk factor of cardiovascular diseases and it is not necessary to discuss the impact of hypertension on morbidity and mortality.

The diagnosis of Circadian Hyper-Amplitude-Tension (CHAT), however, requires a chronobiologic approach as the exact mathematical procedure for data analysis is a prerequisite. Figure 1 indicates a slight, statistically non-significant increase in risk at the lowest amplitude values for SBP and DBP. At higher amplitudes exceeding the threshold value, the increase in risk is much greater. CHAT can be present in both MESOR-normotensive and MESOR-hypertensive patients. The cardiovascular disease risk associated with CHAT can actually be greater than that related to MESOR-hypertension in some outcome studies (Table 1), notably in terms of cerebral ischemic events and nephropathy. One can speculate that since in most cases, CHAT is accompanied by MESOR-hypertension, it is possible that normal or slightly

below normal pressure at night with increased pressure in the blood vessels by day can be more devastating than the sustained increased pressure during both day and night to which blood vessels may adapt by a structural reconstruction of the vascular wall.

High pulse pressure is a result of increasing the augmentation index, which is a measure of the stiffness of the aorta. Increased stiffness of the aorta is a known risk factor for cardiovascular mortality and morbidity.

Reduced heart rate variability is the expression of increased sympathetic and/or decreased parasympathetic activity. Increased sympathetic activity does not harm a healthy heart, but in the heart affected by ischemia, it can induce variations in the speed of conduction in neighboring areas of the ventricular myocardium and lead to the creation of arrhythmogenic bearings, thereby causing ventricular fibrillation and sudden cardiac death.

As described herein, ABPM combined with a chronobiologic interpretation of the data, for self-help by the patient can greatly refine the diagnosis without necessarily increasing the cost of healthcare. Recommendations have been made in a so-called Brno consensus, which was signed by colleagues participating in the BIOCOS project [12], from Brno, from Graz (Austria) and from Minnesota, on the occasion of regular meetings on "Noninvasive Methods in Cardiology" held at Masaryk University, in Brno, Czech Republic. Franz Halberg provided crucial information documenting the merits of assessing the circadian variations in BP and HR; he contributed invaluable data showing the need to measure around the clock for more than one or two days; and he developed the methodology to analyze the data and to interpret them in the light of time-specified reference values, qualified by gender and age. It will have to be up to us to follow in his footsteps to make sure a chronobiologic approach enters the mainstream of medicine.

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Extended Consensus on Guidelines for Assessment of Risk and Management of Hypertension: A Scientific Statement of the International College of Cardiology – Thank You, Dr. Franz Halberg.

Ram B Singh[1], Krasimira Hristova[2], Daniel Pella[3], Jan Fedacko[4], Adarsh Kumar[5], Hilton Chaves[6], Ratindra Nath Mondal[7], Branislav Milovanovic[8], |Germaine Cornelissen[9], Othild Schwartzkopff[9], Franz Halberg[9], and DW Wilson[9]

[1]Halberg Hospital and Research Institute, Moradabad, India
[2]Department of Noninvasive Functional Diagnostics and Imaging, University National Heart Hospital, Sofia, Bulgaria
[3]Faculty of Medicine, P.J. Safarik University, Kosice, Slovakia
[4]Centre of Excellence for Atherosclerosis Research, Louis Pasteur University Hospital, Faculty of Medicine, PJ Safarik University, Kosice, Slovakia
[5]Cardiology Department, Governmental Medical College/GND Hospital, Punjab, India
[6]Faculdade de Medicina, Universidade Federal de Pernambuco, Recife, Brazil
[7]Department of Medicine, Rangpur Community Medical College, Rangpur, Bangladesh
[8]Department of Cardiology, University Clinical Center BezanijskaKosa, and Medical Faculty, University of Belgrade, Serbia
[9]Halberg Chronobiology Center, University of Minnesota, Minneapolis, MN, USA
[10]School of Medicine, Pharmacy and Health, Durham University,UK

Abstract

In most of the guidelines by various agencies, a widespread belief exists that underlying usual blood pressure can alone account for all blood pressure related risk of vascular events and for the benefits of anti-hypertensive drugs. This view may not be correct because it does not consider total risk. Around-the-clock ambulatory blood pressure monitoring is necessary for at least 7 days to better assess risk related to blood pressure and blood pressure variability. Reference limits for blood pressure are currently based mostly on cohort studies and on controlled drug trials conducted among hypertensive patients. This must be changed. Using fixed limits for all adults 18 years and older (or in just 2 age groups) should be replaced by time-specified limits qualified by gender, age, and ethnicity, to be derived from clinically healthy populations, as done on too small a scale within the project on The BIOsphere and the COSmos (BIOCOS). This special paper highlights Vascular Variability Disorders (VVDs), which include, with MESOR-hypertension, Circadian Hyper-Amplitude-Tension (CHAT), ecphasia, ecfrequentia, excessive pulse pressure, excessive pulse pressure product, and a deficient heart rate variability. Anti-hypertensive drugs with their bioavailability as well as bioactivity have to be optimized by chronotherapy to improve benefit and reduce side effects, as documented by a great scientist and human being, Professor Franz Halberg, an exceptional, remarkable man, the father of chronobiology, who introduced the concepts of chronopharmacology and chronotherapy.

Keywords*:* Bioactivity, bioavailability, chronotherapy, anti-hypertensive drugs.

Introduction

The International College of Cardiology, in its meeting (the 7th International Congress on Cardio-vascular Diseases (7th ICCD, Oct 24-26,2013, Sofia,

Bulgaria, www.iccsk.bizpa.in) reiterates that treatment decisions for patients should be dictated by their overall level of risk [1-3]. Such a holistic approach includes an assessment of other cardiovascular risk factors, asymptomatic organ damage, the presence or absence of diabetes, overt cardiovascular disease, or chronic kidney disease [3]. Hypertension is the most prevalent treatable vascular risk factor. However, it is not clear how it causes end-organ damage and vascular events.

A widespread but questionable [3-6] belief exists that underlying usual blood pressure can alone account for all blood pressure related risk of vascular events and for the benefits of anti-hypertensive drugs. This conventional notion has come to underpin all major clinical guidelines regarding diagnosis and treatment of hypertension which are open to bias due to the influence of the drug industry weighing on the results of drug trials. There are other potentially important risk markers which have not been examined widely. These are Vascular Variability Disorders (VVDs) or persisting alterations of the circadian blood pressure and heart rate patterns [6].

These VVDs as well as variability in clinic blood pressure measurements or maximal blood pressure values reached have been neglected, and effects of anti-hypertensive drugs on such measures are largely unknown. The majority of the guidelines recommend that episodic hypertension not be treated, and the potential risks of residual variability in blood pressure in treated hypertensive patients have been ignored. The morning rise in blood pressure, an acceptable circadian pattern (Figure 1), as well as influences from lifestyle (nutrition, exercise) and the environment (terrestrial and space weather) should be considered in the guidelines [2, 7-11]. Whereas limitations of the usual blood pressure hypothesis and importance of blood pressure variability, instability, and episodic hypertension have been reported by a few experts, ambulatory blood pressure monitoring is only recommended for special cases and is usually limited to 24 hours. Moreover, the analysis of these data remains limited to the computation of 24-hour, daytime and nighttime mean values and their standard deviation. Occasionally, ambulatory blood pressure monitoring is used [2,12-14]. A day-night ratio may be computed for classification in terms of dipping, but this approach has been shown to be inferior to the assessment of the circadian rhythm characteristics interpreted in the light of reference values qualified by gender and age [15]. This short review discusses shortcomings of the usual blood pressure hypothesis, provides background to accompanying reports on the importance of blood pressure variability in predicting risk of vascular events and in accounting for benefits of anti-hypertensive drug therapy suggested by various guidelines.

The National Heart, Lung and Blood Institute Initiative (NHLBI)

The *Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure* released the first JNC report in 1976, followed by several additional JNC reports, including the JNC 7 [16] released in 2003, and most recently the JNC 8 released in 2013 [11]. The NHLBI has a long history of developing clinical practice guidelines, particularly those addressing cardiovascular risk factors. The Adult Treatment Panel (ATP) released clinical guidelines on the *Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults* in 1988, followed by ATP II and III, with an update in 2004. The *Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults* were released in 1998. These guidelines have been extremely successful in calling attention to, and improving care for, the cardiovascular risk factors of hypertension, high blood cholesterol, and obesity.

The NHLBI has now embarked upon a new vision for guidelines regarding the prevention of cardiovascular diseases (CVDs). The guidelines need modification by using an integrative approach and employing new state-of-the-art evidence-based methods. The International College of Cardiology aims at modifying these guidelines according to the culture and health behavior in other countries.

In 2005, the NHLBI convened to seek input for developing the next generation of clinical guidelines on cardiovascular risk. Recommendations included maintaining risk-factor-specific clinical guidelines as well as establishing a process to integrate the science and clinical recommendations for reduction of cardiovascular risk. In 2006, the NHLBI convened a meeting revisiting recommendations for creating

integrated guidelines to address real-world clinical issues, attending to various approaches for risk assessment, including unified lifestyle recommendations, and focusing on implementation aspects in clinical practice. In 2007, the NHLBI convened another meeting to obtain more specific input on the process, approaching both the public and private sectors, including representatives from key stakeholder organizations, researchers, and practitioners.

The most recent version of the guidelines is based on randomized, controlled trials, the quality of the evidence and recommendations graded based on their effect on important outcomes [9, 11]. The guidelines advocate that subjects aged 60 years and older be treated to achieve a blood pressure not exceeding 150/90 mmHg, while younger patients (30-59 years of age) should have a diastolic pressure not exceeding 90 and a systolic pressure not exceeding 140 mmHg, although evidence for reducing the systolic blood pressure below this level is not very strong. These thresholds and goals are recommended for adult patients with hypertension, with kidney disease and/or with diabetes mellitus. There is some evidence to support that treatment with angiotensin-converting-enzyme inhibitors or angiotensin-receptor blockers may provide greater protection among these patients [11]. It is suggested by the ICC experts that these agents may be preferred as the primary line of treatment among populations predisposed to insulin resistance and the metabolic syndrome. It has been emphasized that these guidelines are not a substitute for clinical judgment and decision about care of the individual patient, which should rest on clinical characteristics and overall risk [11].

The European Society of Cardiology Guidelines

Recently the European Society of Cardiology guidelines advocated that systolic blood pressure values of 140 mmHg and above should be treated [10]. The result of these deliberations is the current major effort at the NHLBI in leading the development of a set of evidence-based, comprehensive clinical guidelines for cardiovascular risk reduction in adults. The guidelines will be principally aimed at primary care practitioners and their patients to assist adults in reducing their cardiovascular risk. The guidelines should also be valuable to cardiovascular specialists and their patients in the United States as well as in other countries where the guidelines can be modified. The process will also identify areas where additional research is needed to obtain evidence that can inform practice.

Blood Pressure Categories

All patients with high blood pressure can be stratified into four categories. According to the 2007 guidelines of the European Society of Cardiology, they are:

High-normal blood pressure (130-139 systolic or 85-89 mmHg diastolic),

Grade 1 hypertension (140-159 systolic or 90-99 diastolic mmHg),

Grade 2 hypertension (160-179 systolic or 100-109 mmHg diastolic),

Grade 3 hypertension ($\geq$180 systolic or $\geq$110 mmHg diastolic).

The presence or absence of other cardiovascular risk factors or organ damage/disease should then be factored into treatment decisions for the management of high blood pressure (a full risk-assessment algorithm is included in the guidelines). Diet and lifestyle recommendations for lowering blood pressure are as important as drug therapy [3, 17-19]. Recommending salt intake of approximately 5-6 g per day, in contrast to a typical intake of 9-12 g per day, may be useful to some patients [10], although the blood pressure response to salt restriction should be assessed on an individualized basis, since in some patients, salt reduction can actually increase blood pressure, as shown by Franz Halberg as well as others [20-24]. Conventional (non-chronobiologic) studies indicate that, on average, a decrease in salt intake to 5 g per day can decrease systolic blood pressure about 1-2 mmHg in normotensive individuals and 4-5 mmHg in hypertensive patients. Since the optimal bodymass index (BMI) is not known, the guidelines recommend getting BMIs down to 25 kg/m^2 and reducing waist circumferences to less than 102 cm in

men and 88 cm in women. A weight loss of about 5 kg can decrease systolic blood pressure by as much as 4 mmHg, while aerobic endurance training in hypertensive patients can reduce systolic blood pressure 7 mmHg, independently of any decrease in body weight [10, 17-19]. Exercise also benefits brain function by increasing brain derived neurotrophic factor and neuronal growth factor. Exercise training particularly in the morning also has beneficial effects on HRV, VEGF and HDL, which protect against atherosclerosis and neuronal degeneration [18].

Extended Consensus Proposed by Franz Halberg and Biocos

Monitoring blood pressure and heart rate around the clock for at least 7 days at the outset is recommended for everybody, not just special cases. As reviewed in detail elsewhere in this journal [6], alterations in the variability of blood pressure and/or heart rate are also associated with an increased cardiovascular disease risk, as shown in several outcome studies (for review see [25]). These Vascular Variability Anomalies (VVAs) include, with MESOR-hypertension, an excessive circadian amplitude of blood pressure (CHAT, short for Circadian Hyper-Amplitude-Tension), ecphasia (circadian phase of blood pressure but not of heart rate occurring at an odd time), ecfrequentia (statistically significant departure of the circadian period from 24 hours), excessive pulse pressure, excessive pulse pressure product, and a deficient heart rate variability.

These VVAs are identified in the light of reference values qualified by gender and age whenever possible, awaiting further refinement in terms of ethnicity, reference values being determined on the basis of around-the-clock ambulatory blood pressure records from clinically healthy subjects [26]. VVAs occurring transiently may be indicative of the presence of loads [27]. When they persist in repeated week-long records in the absence of loads, they become VVDs. When two or more VVDs coexist, they are referred to as Vascular Variability Syndromes (VVSs). (Figure 2)

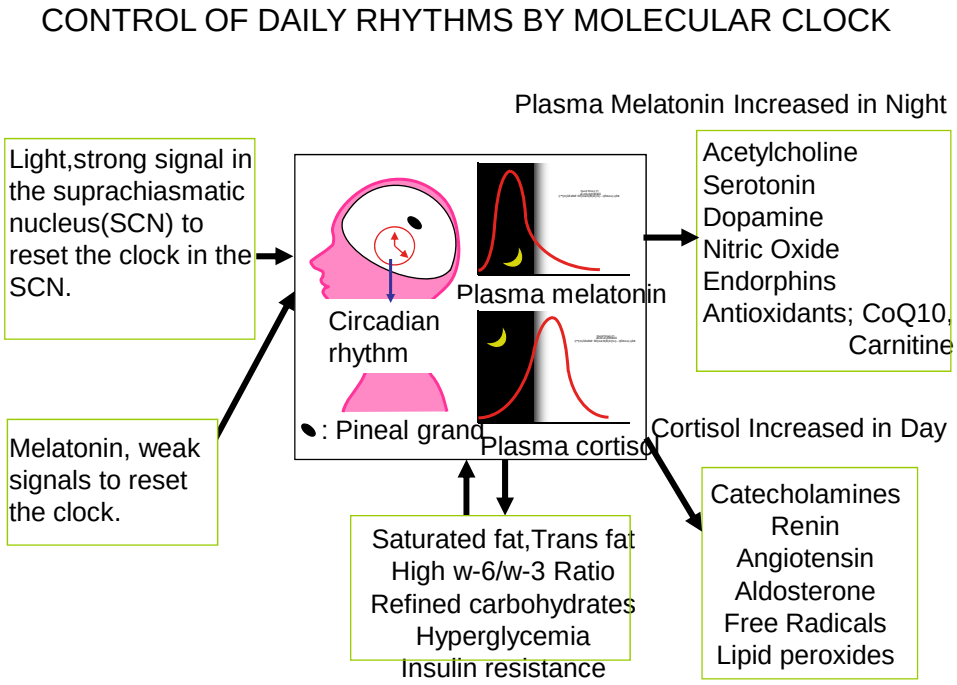

Figure 1. Some input and output signals underlying the circadian system.

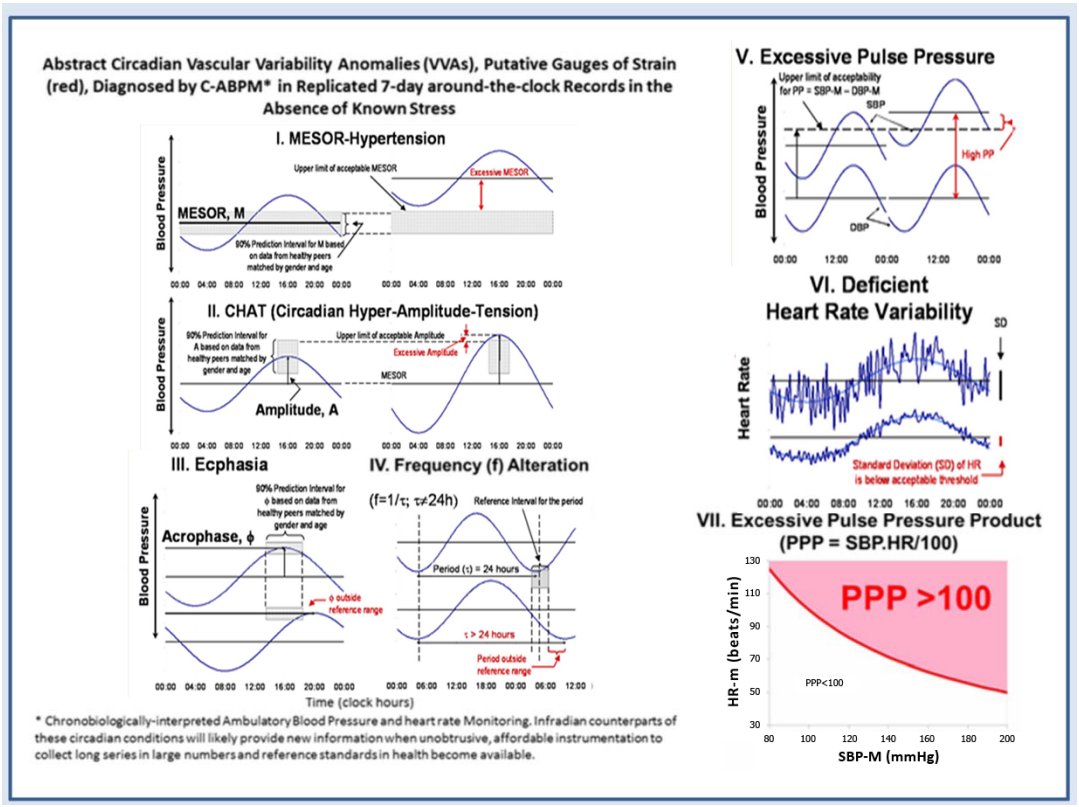

Figure 2. Showing vascular variability disorders.

VVAs (VVDs) cannot be screened for by means of casual single measurements in the clinic. Around-the-clock measurements need to be analyzed chrono-biologically by sphygmochron [6, 28]. This analysis relies on a two-pronged approach: parametrically, a 2-component model consisting of cosine curves with periods of 24 and 12 hours is fitted by least-squares to the data [29-31] to better approximate the circadian waveform [32, 33], nonparametrically the data are stacked over an idealized 24-hour day and interpreted in the light of time-specified reference values qualified by gender and age to determine the percentage time elevation, the amount of excess and the time when most excess occurs [6, 28]. For most of the blood pressure range, there is a linear relationship between cardiovascular disease risk and the blood pressure MESOR. This is not the case for some other endpoints of blood pressure and heart rate variability underlying VVAs (VVDs). The relationship between cardiovascular disease risk on the one hand and the circadian amplitude of blood pressure, the circadian acrophase of blood pressure, and the standard deviation of heart rate on the other hand is nonlinear: risk is increased only once a threshold value is exceeded [34].

VVAs (VVDs) are mostly independent and contribute additively to cardiovascular disease risk [6, 28]. As illustrated in one outcome study, the incidence of morbid events within 6 years of the monitoring session in the presence of uncomplicated MESOR-hypertension (elevated blood pressure) is about 8%. The presence of one, two or three additional VVAs is associated with a sharp increase in the incidence of adverse outcomes, Figure 3 [6, 25].

Screening for VVAs (VVDs) is important because it is possible to treat them, sometimes by only changing the timing of administration of the anti-hypertensive medication [35]. Doing so can more than halve the incidence of strokes and overall cardiovascular mortality [36]. Selecting the most appropriate anti-hypertensive agent [37, 38] and determining the optimal circadian stage of treatment for each patient [39] can avoid causing iatrogenic harm and restore blood pressure and heart rate variability within acceptable limits.

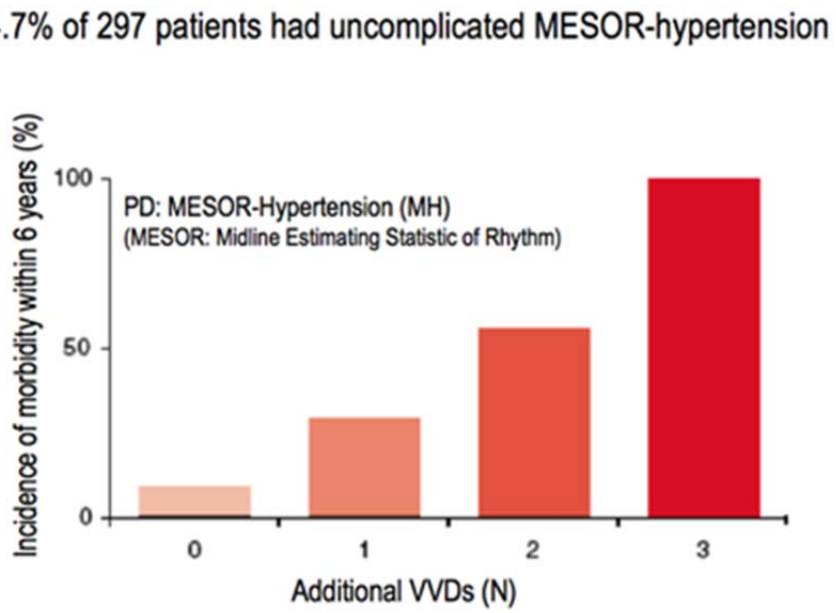

Figure 3. Uncomplicated MESOR-hypertension is associated with about 8% adverse events within 6 years, whereas the presence of 1, 2, or 3 additional Vascular Variability Disorders (VVDs) markedly raises the risk. © Halberg; data from K. Otsuka; analyses by G. Cornélissen [6].

The individualized optimization of treatment timing is rendered feasible by the availability of statistical methods such as parameter tests and the self-starting cumulative sum control chart (for review, see [40]).

Approach to Management

European Union Agenda

It is good to find out the social determinants of health to plan health education by an integrated approach in all of the 52 countries in the European Union (EU) [41-43]. In the EU, 80% deaths occur due to non-communicable diseases (NCDs) including 60-65% due to CVDs [42, 44]. The European countries have witnessed remarkable health gains in those populations which have experienced progressive improvements in the conditions in which people are born, grow, live and work. Despite better health in France, Italy and the Nordic countries, inequities both between and within countries across the 52 Member States of the WHO European region persist [42, 45]. The EU is now in a better position to understand the extent and social causes of these inequities. The EU has established a commission to support the development of the new health policy framework for Europe (Health 2020) because these experts believe that the Western world is not always correct, particularly in matters related to health [23, 45]. The focus of the EU is placed on global evidence regarding socioeconomic policy-related determinants of health across the life course, and on establishing policies [41-45]. This wider vision may ensure that progress can be made in reducing health inequities across all countries of the world. The EU is collaborating with Health Canada, India, China and many other countries to develop a global health agenda, including healthy foods, leading to better health [45-47]. The United Nations are ready to play a central role for all countries, including those with low incomes across the world. The Member States can be encouraged to plan, pursue, and transition to better national healthcare systems to understand that poverty is not the primary cause of death related to NCDs [45-49]. A chrono-nutritional therapeutic approach proposed by Halberg should be planned to achieve food security and greater health equity for future generations in all countries, including Third World countries [48, 49]. This approach may provide guidance as to how best to develop “Functional Food Security” and inexpensive spare time and occupational physical activity across the whole ‘tobacco-free’ world for prevention of NCDs.

International College of Cardiology (ICC 2013)

The ICC reiterates the above mentioned efforts and challenges, which may not be final, because primarily, we should target health behavior and health education about prevention to address all four components of total health, as emphasized by the International College of Cardiology and the International College of Nutrition [3, 17, 19]. This includes the prevention of deaths due to injury as well as improvements in social, mental and spiritual health which appear to be least known to people and experts in the West. Various social markers of health and wealth can influence the levels of health behavior: physical inactivity, dietary patterns, salt intake, alcohol consumption and tobacco use and psychosocial stress are all important factors in the pathogenesis of CVDs [41-48]. Big food and big systems have become common in wealthy countries which are being followed in the developing countries resulting in an increased risk of CVDs [50].

Effective control of NCDs requires a comprehensive approach. There is a need to collaborate between the department of agriculture, food and nutrition, the department of education and the department of sports on the one hand, and transportation and housing on the other hand, in every country. But the United States should take the lead in view of its influence on all other countries. A health promotion policy could thus be made so that students right from play schools to postgraduate colleges as well as citizens working in offices or factories can have the opportunity to get slowly absorbed, micronutrient dense, ω-3 fatty acid rich, ready prepared functional foods, cola drinks/fruit juice as well as fresh foods and spare time physical activity at affordable cost. A public health policy providing tax relief to the food industry, farmers growing healthy foods (fruits, vegetables, seeds and nuts and herbs), food departmental stores and public/private health promotion centers; gymnasiums, yoga and meditation centers, parks and foot paths for cycling and walking may be great steps in a tobacco-free world toward the prevention of NCDs. There is a need to develop more concentrated flavonoid-rich wines, like Chinese wines, to avoid the use of alcohol-rich spirits. These efforts should be started also in the Third World and lower middle income countries, apart from the developed countries.

Emphasis on Halberg's approach, namely chronotherapy with foods, drugs and exercise, deserves renewed interest in view of its promise to decrease cost in both developing and developed countries, because the required quantity of food and drugs could be reduced by up to 50%, while their efficacy could be increased several fold, with fewer and less severe adverse effects [18, 25, 36].This novel approach is likely to contribute to solving the healthcare crisis by placing greater focus on population health [51]. Despite sincere efforts, the current system has failed to achieve targets for health outcomes, eliminate disparities in health and healthcare, and function within a sustainable budget [51]. The role of blood pressure variability and associated risk factors, low antioxidant vitamins, active prayer, fruit and vegetable intake have also been observed in various studies (52-55).Some important issues are:

1. Apart from physical, social, mental and spiritual health, environmental health related to pollution and geomagnetic activity appears to be quite important to achieve total health.
2. The care or management for NCDs is a global problem; hence the Pan American Health Organization has taken new steps to prevent NCDs (http://new.paho.org /hq/index. php? option=com_content) which need further modifications. Research demonstrates that the vast majority needs a model of healthcare that could deliver integrated management of NCDs within the context of primary healthcare, and provides practical guidance for healthcare program managers, policy-makers, and stakeholders on how to plan and deliver high-quality services for people with NCDs.
3. Enacting policies and programs related to food, agriculture, affecting production, trade, manufacturing, labeling, public-private partnerships, taxes and subsidies may be useful. The emerging and dynamic consumer market for healthier food options should be encouraged. Public policies can support consumers in making good nutrition decisions

and reduce future healthcare expenditures in the process.

4. Appropriately applied reductions in salt and replacement of transfat with polyunsaturated fat are among WHO best buys but there should be greater emphasis on slowly-absorbed, ω -3 fatty acids and a flavonoid-rich diet. Such inter-ventions could also contribute to develop-ment goals, such as improving maternal health by reducing conditions such as diabetes and hypertension in pregnancy, with benefits for the offspring as well.
5. For increasing activity, urban planning can increase access to rapid mass transit and safe cycling and walking paths. For example, the use of Transmilenio, rapid mass transit in Santiago, Chile, has been shown to increase the chance of walking for more than 30 minutes a day by 70 percent.

Efforts by the US government to provide funds from health insurance companies is a half hearted approach and should not be adopted by other countries. There is a need for the governments of all countries to collaborate between the Ministry of Health, the Ministry of Education, the Ministry of Sports, the Ministry of Food and Nutrition and the Ministry of Agriculture for working together in policy making to change the health behavior of the populations rather than targeting secondary risk factors of NCDs. It should be reemphasized that poverty is not the cause of emergence of NCDs but poor health behaviors are actual causes of death due to NCDs [43, 48].

In brief, industry and institutions involved in providing physical activity, health foods, stress relief by yoga and meditation, tobacco and alcoholism cessation, moderate wine drinking, decreasing pollution should be encouraged by giving them tax relief by the government to encourage healthier lifestyles for the prevention of NCDs. These measures may bring about an apparent decrease in the income to governments but should be cost-effective in the long run by decreasing healthcare budgets and by increasing work efficiency of every country.

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