

Chronocardiology and Chronotherapy

Ram B Singh, Theodora H Darlenska, Krasimira Hristova, Kuniaki Otsuka, Jan Fedacko, Daniel Pella, Branislav Milovanovic, Ranjana Singh

Ram B Singh, Halberg Hospital and Research Institute, Moradabad, India

Theodora H Darlenska, Department of Pharmacology, Medical University in Sofia

Krasimira Hristova, National University Heart Hospital, Sofia, Bulgaria

Kuniaki Otsuka, Department of Internal Medicine, Tokyo Women's Medical University, Medical Center East, Tokyo, Japan

Jan Fedacko, Daniel Pella, PJ Safaric University, Kosice, Slovakia
Branislav Milovanovic, University Clinical Center Bezanijnska Kosa, University of Belgrade, Serbia; Department of Biochemistry, KG Medical University, Lucknow (UP), India

Ranjana Singh, Ranjana Singh, Department of Biochemistry, KG Medical University, lucknow, India

Correspondence to: Ram B Singh, MD, Halberg Hospital and Research Institute, Civil Lines, Moradabad-10(UP)244001, India.

Email: rbs@tsimtsoum.net

Telephone: +915912417437

Fax: +915912417437

Received: December 24, 2013

Revised: February 14, 2014

Accepted: February 20, 2014

Published online: March 10, 2014

therapy

Singh RB, Darlenska TH, Hristova K, Otsuka K, Fedacko J, Pella D, Milovanovic B, Singh R. Chronocardiology and Chronotherapy. *Journal of Cardiology and Therapy* 2014; 1(2): 31-36 Available from: URL: <http://www.ghrnet.org/index.php/jct/article/view/617>

INTRODUCTION

Variability in blood pressures and myocardial function, heart rate, according to time structure particularly on a 7-days scale, has been ignored by most of the experts, despite repeated attempts by Franz Halberg who is considered a pioneer on chronobiology and chronotherapy^[1-3]. The role of variability in blood glucose causing increased risk of target organ damage is now well known. However, recently, a few experts reiterate their viewpoints on various aspects of ambulatory blood pressure (BP) monitoring which appears to be quite interesting, although it is limited 24-hours monitoring^[4]. Extended consensus on need and methodology to detect vascular variability disorders (VVDs), and vascular variability syndromes (VVSs), and the role of brain-body interactions, are recent advances in chronocardiology^[3-6]. It is noteworthy that circadian periodicity in BPs, have become important due to its influence on chrono-cardiovascular dysfunction which may be pivotal in the prevention of cardiovascular diseases (CVDs) (Figure 1 and 2).

CHRONOCARDIOLOGY

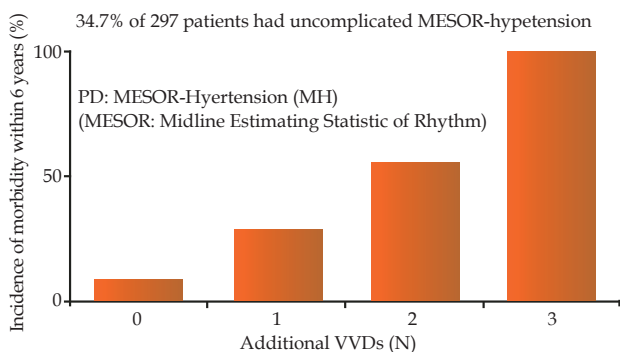
Chrono-cardiology is the science which refers to cardiovascular function and dysfunction according to time structure. Global guidelines for the management of CVDs emphasize that the necessity, choice and intensity of treatment should be determined by the individual's probability of an event (risk) within a given period leading to cardiovascular disease or death (CVD)^[4-6]. Unfortunately, assessment of the cardiovascular risk, which is based on secondary risk factors such as waist circumference and/or body mass index, blood glucose, blood lipids is subject to bias. The presence of associated clinical conditions and/or end-organ damage are not adequately considered. Primary risk factors; age, sex, family history, sedentary behavior, excess of salt and alcohol consumption, tobacco intake, Western diet, mental load are well known risk factors of CVDs^[5-8]. These risk factors can influence circadian

ABSTRACT

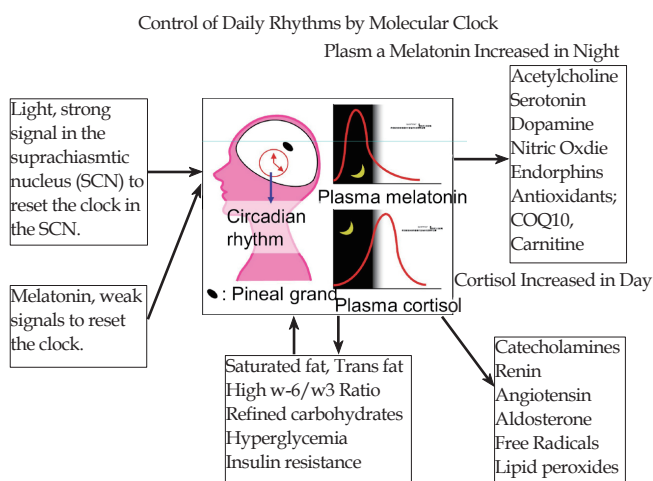
Chrono-cardiology is the science which refers to cardiovascular function and dysfunction according to time structure. Guidelines from various agencies for the management of cardiovascular diseases (CVDs) emphasize that the necessity, choice and intensity of treatment should be determined by the individual's probability of an event (risk) within a given period which predisposes to CVD and deaths. There is little consideration to chronotherapy based on data collected, for example, during the 24 hours, which can fail to detect vascular variability disorders not yet popularly known among cardiologists. A knowledge of chronocardiology and chronotherapy may be useful in reducing the dose of the agent as well as its adverse effects while providing several fold greater benefit, thereby optimising therapeutic efficacy and toxicity.

© 2014 ACT. All rights reserved.

Key words: Drug therapy; Lifestyle; Chronobiology; Circadian



Figures 1 MESOR Hypertension (Halberg *et al*^[14] 2010).



Figures 2 Effect of environmental factors in the pathogenesis of circadian rhythms.

rhythms of cardiac events as well as other biological functions which have a periodicity. These physiological functions and their variability according to time structure, may be used as guideline for chronotherapy.

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 (diurnal)^[11] and constant dim light (circadian) conditions^[9,10]. In clinical studies, night shift work is known to predispose CVDs^[11,12]. In a recent study among 14 healthy nursing professionals, aged 20-40 year, performing day and night shifts and 14 subjects as controls, performing day duty were recruited in the study. Circadian patterns of blood pressure and heart rate were evaluated in night shift workers during the work shift (Night & Day shift) and in controls^[12]. Night shift workers showed a very interesting altered circadian pattern of double amplitude when subject went back to the day shift. An extremely significant different pattern of SBP and DBP double amplitude was found between day shift and controls ($p < 0.01$). Night shift work or late sleep may cause 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 and metabolic syndrome^[11,12].

Disruption of the circadian clock within the cardiomyocyte influences myocardial contractile function, metabolism, and gene expression^[13,14]. The role of intrinsic heart circadian clock in the transcriptionally based molecular mechanism in cardiovascular biology is poorly understood^[13]. It is possible that the circadian clock within the cardiomyocyte influences diurnal variations in myocardial biology. In an experimental study, “CCM mice exhibit

normal myocardial contractile function *in vivo*, as assessed by echocardiography”^[14]. There was, as we cite, almost verbatim, Molly Bray *et al* an “attenuation of heart rate diurnal 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. The normal diurnal 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 role of time structure on biochemical and biological function was pointed out by Franz Halberg much before Barter^[15,16]. He went on conducting research starting from molecular medicine to clinical cardiology which is popularly known as chrono-cardiology, the name given by him^[1-3].

CHRONOTHERAPY

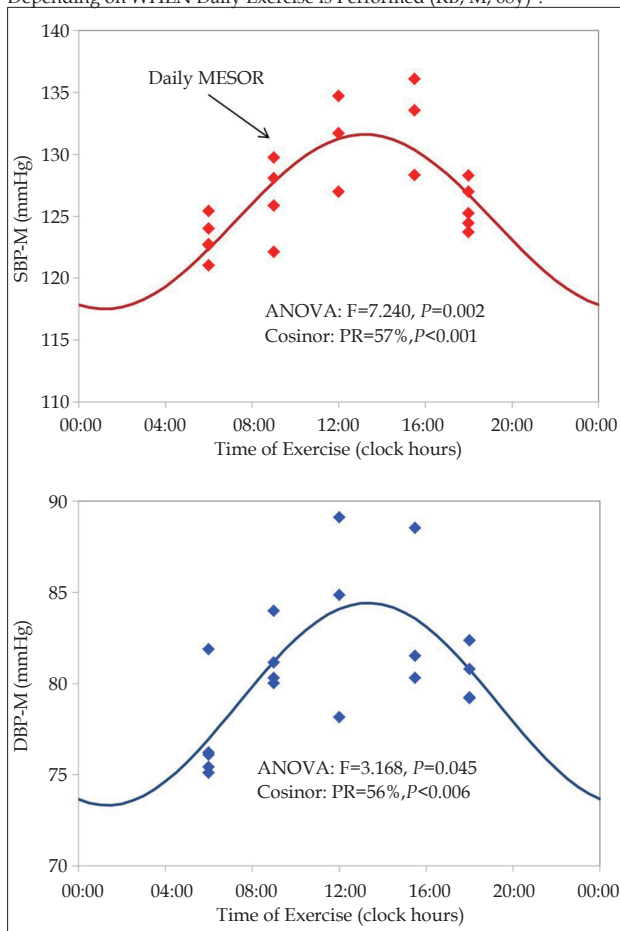
Chrono-therapy is the method of treatment where time of therapy determines the bioavailability and bioactivity of the modality of treatment. The adverse effects of any therapeutic agent can be eliminated or reduced in extent and bioactivity can be increased by the rescheduling the treatment along the 24-hour scale^[17-22]. The treatment may be modifications of routine activities, diet and/or daily exercise and/or staving or antihypertensive medication^[23-25]. 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 [Chronobiologically interpreted automatic ambulatory BP and heart rate (HR) monitoring], wrist activity or a human metabolite timetable^[23]. 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^[26]. 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 CoQ10 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. In an interesting case it has been shown for the first time, how statistically significant results can be obtained by chronotherapy with exercise training, with a simple individualized design, that can be self-applied by everybody to optimize a desired effect by Chronobiologically-interpreted Ambulatory Blood Pressure (BP) and heart rate (HR) Monitoring (Figure 3). A 68-year old internist cardiologist monitored himself at half-hourly intervals, with interruptions, for 3-to-7-day sessions (total about 4 weeks). The exercise timing being kept the same within a given session and changed from one session to another. Exercise training in the morning was associated with lowest BP and HR MESORs (Midline Estimating Statistic Of Rhythm, a rhythm-adjusted mean) as compared to exercise done at mid-day,

late afternoon or in the evening^[25]. It seems that exercise effects are circadian stage- dependent and that exercise at the wrong circadian stages can induce a Vascular Variability Disorder (VVD) has been documented earlier^[5,25].

BLOOD PRESSURE VARIABILITY

Definition of ambulatory BP targets for diagnosis and treatment of hypertension in relation to clinic BP is based on 24-48 hours records which ignores the presence of circa-septan variability in blood pressures as well as VVS and VVDs^[4,27,28]. “The superiority of daytime ambulatory over conventional BP in four populations among 7030 individuals^[28] gave little consideration to night time non-dippers^[4,27]. These studies, although prospective, appear to be only speculations in absence of data on circa-septan increase in blood pressure variability and resultant target organ damage^[1-4]. Given that conventional health care practice is concerned mainly with high BP and given the fact that other VVDs; circadian over-swinging, excessive pulse pressure, deficient heart rate variability, odd circadian blood pressure timing and MESOR hypertension are not diagnosed but contribute several fold greater risk of CVDs and deaths^[1-3]. A recent experimental study published in Nature^[29] suggests that a comprehensive re-examination of circadian behavior and its molecular readouts under simulated natural environmental

Systolic (S) and Diastolic (D) Blood Pressure (BP) Differ Predictably Depending on WHEN Daily Exercise is Performed (RB, M, 68y)*.



* One shoe-size (one arbitrary time) does not fit all.

Figures 3 The same subject exercised at different times after awakening in a schedule of sleep/rest from 22:30 to 06:00, demonstrating a circadian rhythm in response to exercise validated by both cosinor and 1-way analysis of variance (ANOVA). ©Halberg.

conditions will provide a more authentic interpretation compared to that observed in the laboratory which is similar to clinic or home BP measurements. If we want to understand exactly how the circadian clock works and how BP variability (BPV) occurs periodically, we are of the firm opinion that it is going to be very useful to have approaches that observe BP of populations living in natural living environment, which is possible only by 7-days ambulatory BP recording (Figure 2)^[29]. We cannot simply transfer what we know in the clinic or office into natural conditions. Unfortunately, current management of hypertension strongly relies on clinical BP measurement, although potential evidence indicate that measurement of BP outside the clinic by ambulatory BP and/or home BP devices better represents patients actual BP. Most experts feel that there is only limited information of how to include ambulatory BP monitoring, as a stronger predictor of clinical outcomes, into the diagnosis and management of hypertension^[1-7]. This observation is also in agreement with a cross-sectional survey among 6740 subjects, aged 25 years and above, which reported that pre-hypertension and hypertension are more prevalent in India than assumed before and considered to be a big public health issue^[8].

In a more recent sub- study from India involving “209 subjects (142 men and 67 women) aged 42.4±18.0 years, in which BPs records were collected for 3-7 days by ambulatory BP monitors, anticipated relationships were found^[7]. Namely showing increase in the MESOR of SBP, DBP and heart rate, with age (SBP: $r=0.260$, $P<0.001$; DBP: $r=0.269$, $P<0.001$; HR: $r=0.242$; $P<0.005$), as well as with body mass index (SBP: $r=0.232$, $P<0.005$; DBP: $r=0.257$, $P<0.001$). The MESOR of heart rate also decreased with increased activity ($F=5.558$, $P=0.001$). The MESOR of SBP decreased slightly with increased activity, but the relationship was not statistically significant.” Of particular importance was the effect of consumption of fruits, vegetables and legumes (FVL) on the MESOR of heart rate ($r=-0.192$, $P=0.011$). The circadian double amplitude of HR was also found to be higher among Hindus who prayed (11.7 beats/min) as compared to Hindus who did not pray (8.1 beats/min) (Student $t=2.137$, $P=0.035$) indicating that prayer may have beneficial effect on parasympathetic activity.

The effects of autogenic training and antihypertensive agents on circadian and circa-septan variation of blood pressures have been observed^[30], indicating that both methods of treatment may be complimentary to each other. A few studies have reported an association between pre-diabetes and diabetes with abnormal circadian blood pressure variability which supports the role of glycemia in the pathogenesis of VVSs and VVDs^[3,31,32]. It seems that the consensus statement made outlines only limited rationale for the recommended upper limits for daytime, night-time and 24-h SBP/DBP (systolic and diastolic BP) levels determined by ambulatory BP monitoring^[4]. Details specific indications for its use, interpretation of the measurements and potential areas of further research are also open to bias because VVSs and VVDs are likely to be missed (Figure 1)^[3,30]. Franz Halberg the father of chronobiology and chronotherapy has laid down guidelines for Chronobiologically interpreted automatic ambulatory BP and heart rate (HR) monitoring in which he has clearly indicated that monitoring should be done for minimum 7-days, otherwise VVDs are likely to be missed^[33]. The role of various environmental factors including nutritional factors which can influence BPV needs further mention (Figure 2). The current guidelines for the diagnosis and management of hypertension in adults also need greater emphasis on blood pressure variability^[34]. The guidelines incorporate revised ambulatory BP thresholds for the diagnosis and management of hypertension but ignore the existence

of VVSs and VVDs^[3,4,31,32,34].

It is well known that specific features of the 24-h BP pattern are linked to progressive injury of target tissues and risk of CVD events. Several studies have consistently shown an association between blunted asleep BP decline and risk of fatal and nonfatal CVD events. Therefore, most experts emphasize to properly control BP during nighttime sleep as well as during daytime activity. The timing of antihypertensive treatment entails to endogenous circadian rhythm determinants of the 24-h BP pattern^[35]. There have been significant and clinically meaningful treatment-time differences in the beneficial and/or adverse effects of at least six different classes of hypertension medications, and their combinations. Calcium channel blockers (CCBs) are more effective with bedtime than morning dosing, and for dihydropyridine derivatives bedtime dosing significantly reduces risk of peripheral edema. Since renin-angiotensin-aldosterone system is highly circadian rhythmic and activates during nighttime sleep, an evening ingestion of the angiotensin-converting enzyme inhibitors (ACEIs) have been found to exert more marked effect on the asleep than awake systolic (SBP) and diastolic (DBP) BP means. Similarly, the angiotensin-II receptor blockers (ARBs) administered at bedtime, in comparison with morning, ingestion schedule, exert greater hypotensive effect on asleep BP, plus significant increase in the sleep-time relative BPs. There is additional benefit, independent of drug terminal half-life, of converting the 24-h BP profile into a more normal dipping pattern. Such results are also observed for the bedtime versus upon-awakening regimen of combination ARB-CCB, ACEI-CCB, and ARB-diuretic medications.

The chronotherapy of conventional hypertension medications constitutes a new and cost-effective strategy for enhancing the control of daytime and nighttime SBP and DBP levels, normalizing the dipping status of their 24-h patterning, and potentially reducing the risk of CVD events and end-organ injury, for example, of the blood vessels and tissues of the heart, brain, kidney, and retina. However, chronotherapy in consideration of 7-day BP records and considering the time of awakening may double these beneficial effects which has been demonstrated by Franz Halberg in relation to other biomarkers.

CVD morbidity and mortality risk reduction by a bedtime versus upon-awakening treatment schedule has never been evaluated prospectively^[36]. The MAPEC study was specifically designed to test the hypothesis that bedtime chronotherapy with ≥ 1 hypertension medications exerts better BP control and CVD risk reduction than conventional therapy, i.e., all medications ingested in the morning. A total of 2,156 hypertensive subjects, 1,044 men/1,112 women, 55.6 \pm 13.6 (mean \pm SD) years of age, were randomized to ingest all their prescribed hypertension medications upon awakening or ≥ 1 of them at bedtime. At baseline, BP was measured at 20-min intervals from 07:00 to 23:00h and at 30-min intervals at night for 48h. Physical activity was simultaneously monitored every min by wrist actigraphy to accurately determine the beginning and end of daytime activity and nocturnal sleep. Those patients, ingesting medication at bedtime showed at their last available evaluation significantly lower mean sleep-time BP, higher sleep-time relative BP decline, reduced prevalence of non-dipping (34% versus 62%; $p < 0.001$), and higher prevalence of controlled ambulatory BP (62% versus 53%; $p < 0.001$).

After a median follow-up of 5.6 years, those patients who took ≥ 1 BP-lowering medications at bedtime exhibited a significantly lower relative risk of total CVD events than those ingesting all medications upon awakening [0.39 (0.29-0.51); number of events 187 versus 68; $p < 0.001$]. The difference between the treatment-time groups in the relative risk of major events (including CVD death, myocardial infarction, ischemic stroke, and hemorrhagic stroke) was also highly

statistically significant [0.33 (0.19-0.55); number of events: 55 versus 18; $p < 0.001$]. In conclusion, evening chronotherapy with ≥ 1 BP-lowering medications, compared to conventional upon-awakening treatment with all medications, more effectively improved BP control, better decreased the prevalence of non-dipping, and, most importantly, significantly reduced CVD morbidity and mortality. In diabetes mellitus, non-dipping (<10% decline in asleep relative to awake BP mean), as determined by ambulatory BP monitoring (ABPM), is more frequent which may be associated with increased CVD risk^[31,32,37]. The influence of hypertension treatment-time regimen on the circadian BP pattern, degree of BP control, and relevant clinical and analytical parameters of hypertensive patients with type 2 diabetes evaluated by 48-h ABPM were studied^[37]. This cross-sectional study involved 2429 such patients (1,465 men/964 women), 65.9 \pm 10.6 (mean \pm SD) years of age. Among the participants, 1,176 were ingesting all BP-lowering medications upon awakening, whereas 1,253 patients were ingesting ≥ 1 medications at bedtime. Among the latter, 336 patients were ingesting all BP-lowering medications at bedtime, whereas 917 were ingesting the full daily dose of some hypertension medications upon awakening and the full dose of others at bedtime. Those ingesting ≥ 1 medications at bedtime versus those ingesting all medications upon awakening had lower likelihood of metabolic syndrome and chronic kidney disease (CKD); had significantly lower albumin/creatinine ratio, glucose, total cholesterol, and low-density lipoprotein (LDL) cholesterol; and had higher estimated glomerular filtration rate and high-density lipoprotein (HDL) cholesterol. However, patients taking all medications at bedtime had lowest fasting glucose, serum creatinine, uric acid, and prevalence of proteinuria and CKD. Ingestion of ≥ 1 medications at bedtime was also significantly associated with lower asleep systolic (SBP) and diastolic BP (DBP) means than treatment with all medications upon awakening. Sleep-time relative SBP and DBP decline was significantly attenuated in patients ingesting all medications upon awakening ($p < 0.001$). Thus, the prevalence of non-dipping was significantly higher when all hypertension medications were ingested upon awakening (68.6%) than when ≥ 1 of them was ingested at bedtime (55.8%; $p < 0.001$ between groups), and even further attenuated (49.7%) when all of them were ingested at bedtime ($p < 0.001$). The prevalence of the riser BP pattern, associated with highest CVD risk, was much greater (23.6%) among patients ingesting all medications upon awakening, compared with those ingesting some (20.0%) or all medications at bedtime (12.2%; $p < 0.001$ between groups). The latter group also showed significantly higher prevalence of properly controlled ambulatory BP ($p < 0.001$) that was achieved by a significantly lower number of hypertension medications ($p < 0.001$) compared with patients treated upon awakening. These results indicate that bedtime drug therapy, in conjunction with proper patient evaluation by ABPM to corroborate the diagnosis of hypertension and avoid treatment-induced nocturnal hypotension, should be the preferred therapeutic scheme for type 2 diabetes.

In cardiomyocytes, when a threshold level of reactive oxygen species (ROS) is reached, mitochondrial oscillations are triggered resulting in the cycling of cytoplasmic and mitochondrial proteins between their oxidized and reduced states mainly driven by ROS and the redox potential of the thiols pool^[38]. Since oxidative stress results from an imbalance of ROS, and mitochondria are the main source of ROS produced by the respiratory chain, a circadian activity of ROS may be important in the pathogenesis of myocardial diseases. This biomarker may be used for the treatment of heart failure, myocardial infarction and cardiomyopathy where oxidative stress is an important

determinant of cardiac damage.

In brief, chronocardiology and chronotherapy appear to have great potential but need further studies to provide more evidence to establish their role in the management of CVDs. For example, the potential role that self-sustained ultradian rhythms may have in oscillatory redox mechanisms in cellular coherence and survival (as indicated for molecular and cellular function relaxation times following perturbation) is undoubtedly an area of future research (DWW Personal communication Professor Lloyd). It would seem logical to complement circadian and circaseptan time structures with those of ultradians, as well as some of those possibly from 'solar imprinting' at the other end of the frequency spectrum.

ACKNOWLEDGMENTS

This work was supported by International College of Cardiology and International College of Nutrition. Conflict of interest has not been declared by authors.

REFERENCES

- Halberg F, Cornelissen G, McCraty R, Czaplicki J, Al-Abdulgader AA. Time structures (chronomes) of the blood circulation, population health, human affairs and space weather. *World Heart J* 2012; **3**: 73-114
- Halberg F, Southern RB, Watanabe Y, Hillman D, Best WR, Schwartzkopff O, and Cornelissen G. Signatures of space weather in the ageing human blood circulation. *World Heart J* 2010; **3**: 43-62
- Halberg F, Cornelissen G, Otsuka K, Siegelova J, Fiser B, Homolka P, Sanchez la Pena S, Singh RB and BIOCOS Project. Extended consensus on need and means to detect vascular variability disorders (VVDs), and vascular variability syndromes (VVSs). *World Heart J* 2010; **2**: 279-306
- Head GA, McGrath BP, Mihailidou AS, Nelson MR, Schlaich MP, Stowaser M, Mangoni AA, Cowl, Brown MA, Ruta LA and Wilson A. Ambulatory blood pressure monitoring in Australia: 2011 consensus position statement. *J of Hyper* 2012; **30**: DOI:10.1097/HJH.0b013e32834de621
- Singh RB and Indian Consensus Group. Prevalence and prevention of hypertension, diabetes mellitus, and coronary artery disease in India. A scientific statement of the Indian Society of Hypertension, International College of Nutrition and International College of Cardiology. *World Heart J* 2010; **2**: 31-44
- Singh RB, De Meester F, Wilczynska A, Wilson DW, Hungin AP. The liver-pancreas and brain connection in the pathogenesis of obesity and metabolic syndrome. *World Heart J* 2010; **2**: 319-326
- Singh RB, Cornelissen G, Otsuka K, et al. Coronary risk factors and ambulatory blood pressures and heart rate in Asian Indians. *The Open Nutr J* 2012; **5**: 79-80
- Singh RB, Fedacko J, Pella D, et al. Prevalence and risk factors for prehypertension and hypertension in five Indian cities. *Acta Cardiol* 2011; **66**: 29-37
- Storch KF, Lipan O, Leykin I, Viswanathan N, Davis FC, Wong WH, Weitz CJ. Extensive and divergent circadian gene expression in liver and heart. *Nature* 2002; **417(6884)**: 78-83
- Durgan D, Hotze M, Tomlin T, Egbejimi O, Graveleau C, Abel E, Shaw C, Bray M, Hardin P, Young M. The intrinsic circadian clock within the cardiomyocyte. *Am J Physiol Heart Circ Physiol* 2005; **289**: H1530-H1541
- Singh RB, Anjum B, Garg R, Verma NS, Singh R et al. Association of circadian disruption of sleep and night shift work with risk of cardiovascular disease. *World Heart J* 2012; **4**: 23-34
- Anjum B, Verma NS, Tewari S, Jain V, Singh R, Bharadwaj S, Naz Q, Mahdi AA, Singh RB, Singh RK. 24-Hour chronomics of ambulatory blood pressure monitoring in rotating night shift workers and controls. *World Heart J* 2012; **4**: 287-296
- Bray MS, Shaw CA, Moore MW, Garcia RA, Zanquetta MM, Durgan DJ, Jeong WJ, Tsai JY, Bugger H, Zhang D, Rohrwasser A, Rennison JH, Dyck JR, Litwin SE, Hardin PE, Chow CW, Chandler MP, Abel ED, Young ME. Disruption of the circadian clock within the cardiomyocyte influences myocardial contractile function, metabolism, and gene expression. *Am J Physiol Heart Circ Physiol* 2008; **294**: H1036-1047
- Durgan DJ, Young ME. The cardiomyocyte circadian clock: emerging roles in health and disease. *Circ Res* 2010; **106**: 647-58. doi: 10.1161/CIRCRESAHA.109.209957
- Halberg F, Visscher MB. Regular diurnal physiological variation in eosinophil levels in five stocks of mice. *Proc Soc Exp Biol (NY)* 1950; **75**: 846-847
- Halberg F, Stephens AN. Susceptibility to quabain and physiologic circadian periodicity. *Proc Minn Acad Sci* 1959; **27**: 139-143
- Halberg F, Haus E, Cardoso SS, Scheving LE, Kühl JFW, Shiotsuka R, Rosene G, Pauly JE, Runge W, Spalding JF, Lee JK, Good RA. Toward a chronotherapy of neoplasia: Tolerance of treatment depends upon host rhythms. *Experientia (Basel)* 1973; **29**: 909-934
- Halberg F. From aniatrotoxicosis and aniatrosepsis toward chronotherapy: Introductory remarks to the 1974 Capri Symposium on timing and toxicity: the necessity for relating treatment to bodily rhythms. In: Aschoff J, Ceresa F, Halberg F, editors. Chronobiological Aspects of Endocrinology. *Stuttgart: F.K. Schattauer Verlag*; 1974. p. 1-34.
- Reinberg A, Halberg F. Circadian chronopharmacology. *Annu Rev Pharmacol* 1971; **2**: 455-492
- Halberg F, Cornelissen G, Singh RB. Timing nutraceuticals. *World Heart J* 2010; **2**: 100-111
- Halberg F, Cornelissen G, Wang ZR, Wan C, Ulmer W, Katinas G, Singh Ranjana, Singh RK, Singh Rajesh, Gupta BD, Singh RB, Kumar A, Kanabrocki E, Sothorn RB, Rao G, Bhatt MLBD, Srivastava M, Rai G, Singh S, Pati AK, Nath P, Halberg Francine, Halberg J, Schwartzkopff O, Bakken E, Shastri VK. Chronomics: circadian and circaseptan timing of radiotherapy, drugs, calories, perhaps nutraceuticals and beyond. *J Exp Therapeutics Oncol* 2003; **3**: 223-260
- Kasukawa T, Sugimoto M, Hida A, Minami Y, Mori M, Honma S, Honma K-i, Mishima K, Soga T, Ueda HR. Human blood metabolite timetable indicates internal body time. *PNAS Early Edition* 2012. <http://www.pnas.org/content/early/2012/08/22/1207768109>. 6 pp.
- Cornelissen G. When you eat matters: 60 years of Franz Halberg's nutrition chronomics. *The Open Nutr J* 2012; **4**: 16-44
- Singh RB, Singh AK, Sharma JP, Singh RK, Kumar A, Rastogi SS, Singh Garima, Kartikey K, Singh S, Fedacko J, Pella D, De Meester F, Wilczynska A, Wilson DW. Nutrition in chronocardiology: we are indebted Professor Franz Halberg. *The Open Nutra J* 2012; 45-65
- Singh RB, Halberg F, Cornelissen G, Siegelova J, Hristova J, Toda E, Takahashi T, Jan Fedacko J, Otsuka K. Personalized circadian timing of exercise. *World Heart J* 2013, **5**: 79-90
- Darzenska TH, Singh RB, Hristova K, Milovanvic B, Fedacko J, et al. timing of statin therapy may eliminate its toxicity and increase its bioactivity. *World Heart J* 2013; **5**: (in press)
- Head G, Mihailidou A, Duggan K, Beilin L, Berry N, Brown M, et al. Definition of ambulatory blood pressure targets for diagnosis and treatment of hypertension in relation to clinic

- blood pressure: prospective cohort study. *BMJ* 2010; **340**: c1104
- 28 Hansen TW1, Kikuya M, Thijs L, Björklund-Bodegård K, Kuznetsova T, Ohkubo T, Richart T, Torp-Pedersen C, Lind L, Jeppesen J, Ibsen H, Imai Y, Staessen JA; IDACO Investigators. Prognostic superiority of daytime ambulatory over conventional blood pressure in four populations: a meta-analysis of 7030 individuals. *J Hypertens* 2007; **25**:1554-1564
 - 29 Vanin S, Bhutani S, Montelli S, Menegazzi P, Green EW, Pegararo M, Sandrelli F, Costa R, Kyriacou CP. Unexpected features of Drosophila circadian behavioural rhythms under natural conditions. *Nature* 2012 Apr 4; **484(7394)**: 371-375
 - 30 Watanabe Y, Cornélissen G, Watanabe M, Watanabe F, Otsuka K, Ohkawa S-i, Kikuchi T, Halberg F. Effects of autogenic training and antihypertensive agents on circadian and circaseptan variation of blood pressure. *Clin Exp Hypertens* 2003; **25**: 405-412
 - 31 Gupta AK, Greenway FL, Cornélissen G, Pan W, Halberg F. Pre-diabetes is associated with abnormal circadian blood pressure variability. *J Human Hypertension* 2008; **22**: 627-633 doi:10.1038/jhh.2008.32.
 - 32 Sánchez-de la Peña S, Gonzalez C, Cornélissen G, Halberg F. Blood pressure (BP), heart rate (HR) and non-insulin-dependent diabetes mellitus (NIDDM) chronobiology. *Int J Cardiol* 2004; **97 (Suppl 2)**: S14
 - 33 Halberg F, Cornélissen G, Hillman D, Beaty L, Hong S, Schwartzkopff O, Watanabe Y, Otsuka K, Siegelova J. Chronobiologically interpreted ambulatory blood pressure monitoring in health and disease. *Global Advances in Health and Medicine* 2012; **1(2)**: 66-123
 - 34 James PA, Carter BL, Oparil S, et al. 2014. Evidence based guideline for the management of high blood pressure in adults . Report from panel members appointed to the 8th Joint national committee. (JNC-8). *JAMA Online*, December 18,2013,doi:10.1001/jama.2013.284427.
 - 35 Hermida RC, Ayala DE, Fernández JR, Mojón A, Smolensky MH, Fabbian F, Portaluppi F. Administration-time differences in effects of hypertension medications on ambulatory blood pressure regulation. *Chronobiol Int* 2013 Mar; **30(1-2)**: 280-314. Epub 2012 Oct 19
 - 36 Hermida RC, Ayala DE, Mojón A, Fernández JR. Influence of circadian time of hypertension treatment on cardiovascular risk: results of the MAPEC study. *Chronobiol Int.* 2010 Sep; **27(8)**:1629-1651. doi: 10.3109/07420528.2010.510230
 - 37 Moyá A, Crespo JJ, Ayala DE, Ríos MT, Pousa L, Callejas PA, Salgado JL, Mojón A, Fernández JR, Hermida RC, et al. Effects of time-of-day of hypertension treatment on ambulatory blood pressure and clinical characteristics of patients with type 2 diabetes. *Chronobiol Int* 2013 Mar; **30(1-2)**: 116-131. Epub 2012 Oct 25
 - 38 Lloyd D, Cortassa S, O'Rourke B, Aon MA. What yeast and cardiomyocytes share: ultradian oscillatory redox mechanisms of cellular coherence and survival. *Integr Biol (Camb)* 2012 January; **4(1)**: 65-74
- Peer reviewer:** Ramon C Hermida, Professor, Bioengineering & Chronobiology Labs, E.I. Telecomunicación, University of Vigo, Vigo 36210, Spain.