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# World Heart Journal

Volume 1, Number 1, 2008

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## **WORLD HEART JOURNAL**

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There are hardly 10 good journals of Cardiology, which cater to several million cardiovascular scientists and physicians. A lot of material is either not published or published in a substandard manner. Topics to be covered in the WHJ include the following: Epidemiology and Prevention, Chronocardiology and Chronomics, Nutrition and Lifestyle in CVD, Clinical Cardiology, Cardiovascular Sciences (Molecular Cardiology : biochemistry and biology), Hypertension, Coronary artery disease, Pharmacotherapy, Electrophysiology, Echocardiography, Nuclear Cardiology, Pediatric Cardiology, Geriatric Cardiology, CVD in women, Cardiac Rehabilitation and Prehabilitation, as well as Interventional Cardiology and Cardiac surgery.

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## EDITORIAL

# Introducing a Journal and its Editor-in-Chief

**Franz Halberg and Germaine  
Cornélissen**

Halberg Chronobiology Center, University  
of Minnesota, Minneapolis, MN, USA

In the issues to follow, a World Heart Journal could usher in a new health care. It can live up to the "new" in this "world". With respect to content, it could deal with new vascular variability disorders, e.g., Figure 1, Sections IIA-C, and Figure 2. As to scope, altered dynamics in blood pressure (BP) and heart rate (HR) constitute a worldwide problem as pre-hypertension and pre-diabetes, as a pre-metabolic syndrome (1-8), occurring in the currently neglected, and hence, to many, "new" normal range, that can be split chronomically, that is time-structurally. The journal can draw attention to predictable rhythms in everyday physiology with treatable alterations that represent risks greater than hypertension, Figure 1. About-daily and about-yearly rhythms, photic and thermic signatures of day and night and of the seasons, are modulated by and, with advancing age, in part replaced by non-photic competing signatures of the sun and the earth, a concern linked to weather in extra-terrestrial space (9-13).

By teaching automated self-surveillance, a new World Heart Journal can serve the care provider and, directly, the care recipient. All it has to do is help 1) replace the question "What is the ('true', or rather imaginary) BP, HR, ...?" by a question regarding variabilities of these and other body functions that are rightly called **variables**, and 2) provide the means to assess alterations of variability, in the light of gender, age and preferably ethnicity-specified reference values, Figures 1-3. Everyone can benefit from the mapping of chronomes (time structures) in us and around us, by diagnosing chronomically abnormal patterns in a person's BP and/or HR and by restoring healthy chronomes with timed treatment (chronotherapy). By assessing, in populations, the wide spectrum of rhythms in morbidity and mortality that are influenced by space weather as well as by local climate, prophylactic interventions on a population basis may become possible (12).

Chronomics complements chronobiology, the study of the mechanisms of biological time structures, Figure 4. For this purpose, chronomics also maps environmental along with biological rhythms. By extending the perspective from an organism as such to its ambiance near and far, chronomics adds a figurative telescopic in time to chronobiology's figurative microscopy in time. The opportunity to treat an elevated risk of developing cardiovascular disease, such as circadian BP overswinging, while all data are in the "normal range" is an immediate benefit documented worldwide in Figures 1-3.

The treatment as well as the detection of risk, called prehabilitation, can be made known for care providers, notably upstream, in medical schools. Everybody interested in self-care could also be informed and motivated by this journal to implement prehabilitation him/herself. Chronomics is a practicable cost-effective complement to investments into rehabilitation after end-organ damage or after an adverse event, such as a myocardial infarction (MI) or brain stroke has occurred. The treatment of high BP also needs more than spotchecks during waking, when, in a given patient, unknown to his care provider, during 11 consecutive days the diastolic BP can reach about 120 mm Hg each night, while values during office hours remain within conventional limits, as it did in a documented case, Figure 1, Section I. Much more important for primary prevention is the availability of special time-microscopic methods to quantify earliest alterations of variability, opportunities that cannot be obtained by the unaided eye alone or by day-night ratios (4, 6, 8).

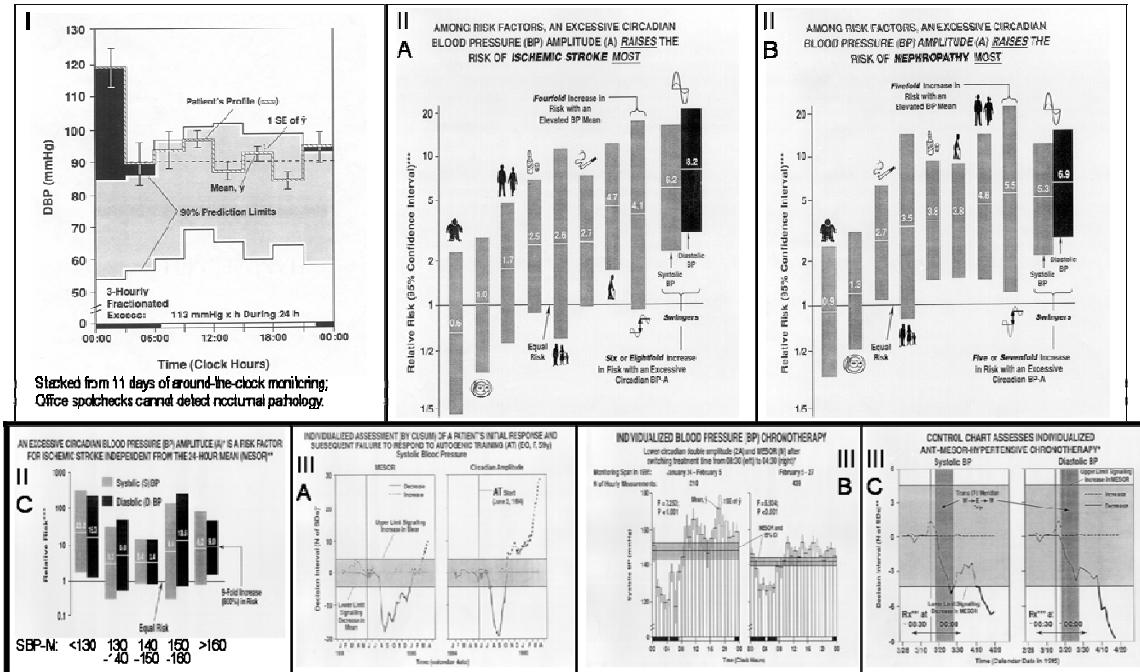
The journal welcomes reports of public as well as professional education to motivate patients to survey their everyday routines by emphasis on nutrition, exercise and other behaviors, notably if they are optimized by timing. BP and HR monitoring for the detection (and treatment) of risk elevation is also important in medicated patients with high BP. By simply changing the timing of conventional medication, monitoring helps adjust the treatment to maximize the hypotensive effect and to minimize risks associated with BP overswinging or other alterations of the BP and/or HR chronomes (1-3, 5, 6). The journal also addresses the timing of nutriceuticals as well as chronobiologic aspects of gene therapy.

Reference to imaginary setpoints is best replaced by measurable changes in chronome characteristics. The journal's topics will include findings on laboratory animals (14), suggesting the possible use of chronomics for rehabilitation, e.g., by considering the role of timing in stem cell collection for cellular cardiomyoplasty and, in the same context, for manipulating, again by timing, various aspects of scar formation. The use of modern telemetry, ongoing routinely in laboratory animals and only in research as yet on humans, may lead to the recognition of the etiology of BP disorders, including overswinging, and thus to the specification of refined treatment options. Chronomics detects not only prehypertension but also a broader premetabolic syndrome, including glucose sensitivity (7, 8). Calling attention to the accumulating evidence in various aspects of chronomics and motivating the population for self-help is a *raison d'être* of this journal.

Prof. Ram Bahadur (R.B.) Singh of Moradabad, India, the editor-in-chief, plays a key role in the BIOCOS project (on The Biosphere and the Cosmos) (15; cf. 9-11), which collects reference values for the foregoing aims, also studying how biological processes are affected by the environment near and far, whenever possible before the dynamics exceed the range of everyday physiology.

Major activities of BIOCOS are the around the clock monitoring and special analyses of BP and HR. Longitudinal studies over decades (in one case for 40 years of manual measurements about 5 times a day or in another case automatically collected half-hourly data for up to 20 years) are routinely complemented by half-hourly around-the-clock surveillance for up to 7 days in various geographical locations, Figure 3, now also including Australia and South America. The records are interpreted in the light of reference standards specified as a function of a broad time structure (including the usually present, often prominent circadian variation), gender and age. For the past several years, in collaboration with BIOCOS, Prof. Singh uncovered associations of circadian characteristics with respect to prayer (15) and nutrition. Some of his other findings, notably in the detection of disorders of BP variabilities (16), are reproduced independently in other BIOCOS centers and have been readily aligned with them, Figure 3.

**Chronomics Detects Nocturnal Escape from Treatment (I), Risk of Stroke and Nephropathy Greater than Hypertension (IIA-B), even in MESOR-Normotension (IIC) and Monitors Transient and/or Lasting Success of Treatment (IIIA-C)\***

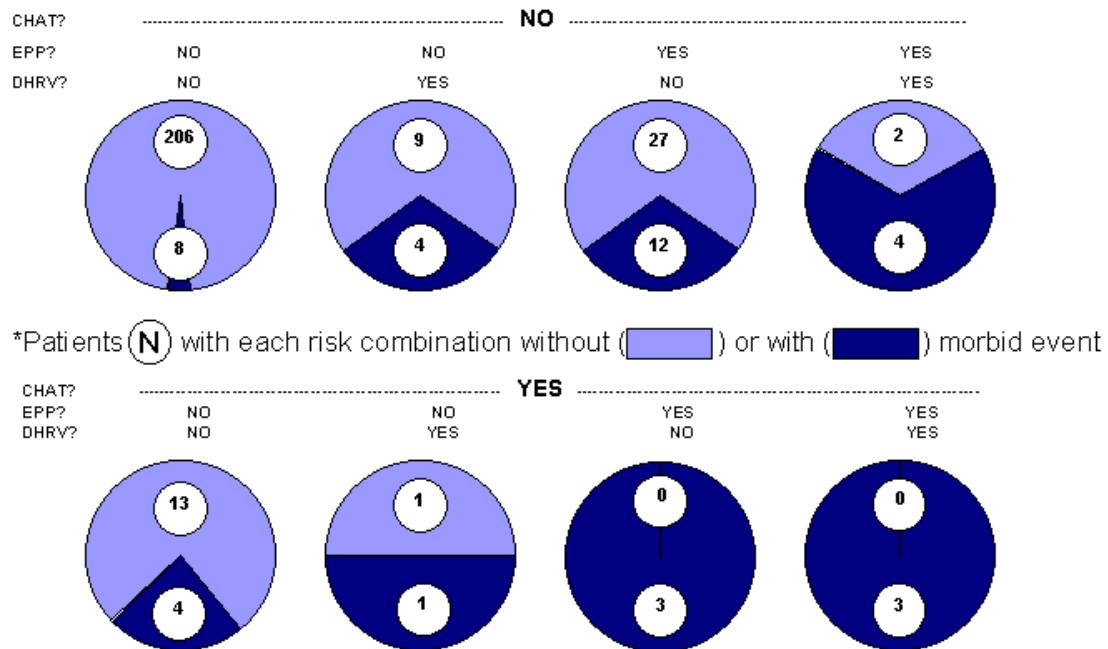


\* During span examined, demonstrating the desirability of lifetime monitoring once abnormality in the normal range is detected (IIC).

Figure 1. Results supporting need for continued surveillance and for chronicomic data analysis. Merits are:

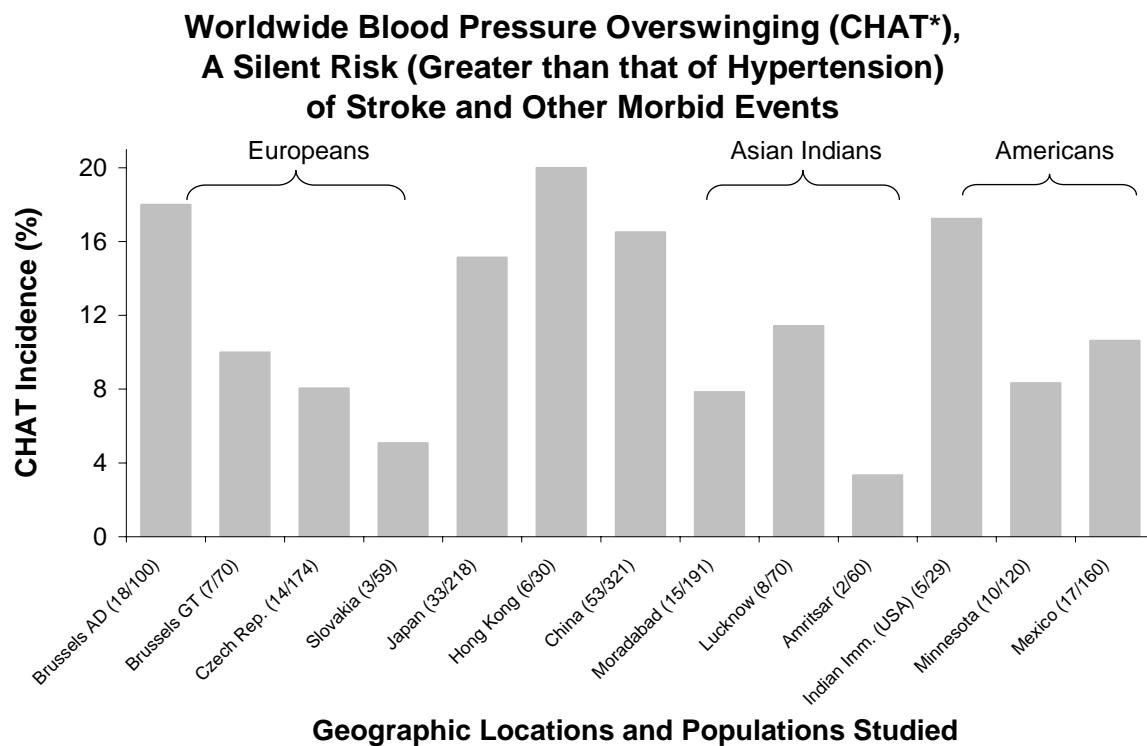
- Detection of abnormality during the night when medication happens to be no longer effective, not seen during office visits in the afternoon when measurements can be acceptable (I; 5, 6);
- Detection of abnormal circadian pattern of blood pressure (CHAT, "overswinging") associated with a risk of cerebral ischemia and nephropathy larger than a number of other risks (including "hypertension") assessed concomitantly (IIA and B);
- CHAT carries a very high risk even among MESOR-normotensives who do not need anti-hypertensive medication (IIC);
- Statistical procedures such as a self-starting cumulative sum (CUSUM), applicable to the individual patient, determine whether an intervention such as autogenic training is effective and for how long the intervention remains effective (IIIA);
- N-of-1 designs for the optimization of treatment timing: the same dose of the same medication can further lower the same subject's blood pressure MESOR and circadian amplitude when the timing of daily administration is changed (IIIB and C), as ascertained by as-one-goes (sequential) testing and parameter tests, procedures applicable to the given individual (3; cf. 39). © Halberg.

**Decreased Heart Rate Variability (DHRV), Circadian Hyper-Amplitude-Tension (CHAT)  
and Elevated Pulse Pressure (EPP) are Separate Cardiovascular Disease Risks\***



\*Results from 6-year prospective study on 297 (adding all Ns) patients classified by 3 risks (8 circles), supported by findings on total of 2,807 subjects for total of over 160,769 sets of blood pressure and heart rate measurements. Data from K Otsuka.

Figure 2. Several conditions related to the variability in blood pressure (BP) and/or heart rate (HR) are each associated with a separate increase in vascular disease risk: a decreased heart rate variability (DHRV), circadian hyper-amplitude-tension (CHAT) and an elevated pulse pressure (EPP). The circadian (or preferably circaseptan) profile gauges EPP as too large a pulse pressure (the difference between systolic [S] BP and diastolic [D] BP, i.e., between the heart's contraction or relaxation, or the extent of change in BP during a cardiac cycle) and DHRV by the standard deviation of HR, in relation to a threshold, (eventually all) in gender- and age-matched clinically healthy peers. Not shown is an abnormal circadian timing of BP but not of HR, circadian BP ephasia, yet another vascular variability disorder, VVD (4). Vascular disease risk is elevated in the presence of any one of these risk factors, and is elevated further when more than a single risk factor (VVD) is present, suggesting that these abnormalities in variability of BP and HR are mostly independent and additive, becoming a vascular variability syndrome, VVS. Abnormalities in the variability of BP and HR, impossible to find in a conventional office visit (the latter aiming at the fiction of a "true" BP), can raise cardiovascular disease risk (gauged by the occurrence of a morbid event like a stroke in the next six years) from 4% to 100%. By comparison to subjects with acceptable BP and HR variability, the relative cardiovascular disease risk associated with DHRV, EPP and/or CHAT is greatly and statistically significantly increased. These risks, silent to the person involved and to the care provider, notably the risk of CHAT, can usually be reversed by chronobiologic self-help, also with a non-pharmacologic approach in the absence of MESOR-hypertension (2). © Halberg.



\* CHAT (Circadian Hyper-Amplitude-Tension) incidence in several geographic locations.

Figure 3. First summary of 191 cases of CHAT in 1602 profiles (many of them covering 7 days) aims at indicating that CHAT is present under various conditions in various populations in many geographic and geomagnetic locations, in the presence or absence of MESOR-hypertension (44). © Halberg.

In cooperating with Prof. Singh, we learned about the many hurdles to be overcome in doing research with relatively limited means. BP and HR records do not always cover 7 days and have interruptions due to problems such as the failure to recharge batteries, as well as to other technical problems with the use of the monitors. Ways to solve problems are invariably sought and a solution is often found eventually. Prof. Singh is involved in many activities, often delegating tasks to his associates or colleagues locally and around the world. In the case of our recent collaboration in BIOCOS, the data are retrieved directly from the monitors electronically and the files are coded, being decoded in our center in Minnesota, where numerical analyses are also carried out. Whenever a question arises, Prof. Singh answers by return mail to our complete satisfaction.

The late V. (Vulimiri) Ramalingaswami, known as Rama (as head of the All-India Institute for Medical Research in New Delhi), and the late B.R. (Bagepalli Ramachandrachar) Seshachar, former

presidents of one of India's two national academies of science, both wanted to bring chronobiology to health care in India, and Rama, later in his life, represented India at the World Health Organization and thus had broader scope. Prof. Singh, by dint of hundreds of already summarized BP and HR profiles, succeeded in implementing what opinion leaders and others sought. Should his recent findings of associations of prayer and diet with desirable aspects of variability be confirmed longitudinally rather than only by a group comparison (15, 16), these non-pharmacological approaches could serve to reduce high vascular disease risk factors, a deficient HR variability and an elevated HR, respectively. The findings on risk are based on a large number of subjects and a large number of data (5, 6). Prof. Singh adds to these findings data from the Indian subcontinent, Figure 3, thus implementing in Moradabad what we achieved neither in Old and New Delhi nor in many developed areas with many more resources. It remains common practice in most research elsewhere to rely on single

measurements of BP as a start (17) or at best on 1- or 2-day around-the-clock profiles (18). Prof. Singh's investigations target routine 7-day half-hourly profiles and by computer analyses of the data, at least in his

research and, we trust, soon in practice, he picks up otherwise undetected variability disorders, that are not recognized by most others.

CHRONOBIOLOGY (CENTER)-SPAWNED TRANSDISCIPLINARY SCIENCES,  
CHRONOMICS AND YET-TO-BE-DEVELOPED CHRONOBIOETHICS,  
AIMING TO SERVE  
INDIVIDUALS' HEALTH, NATIONS' WELL-BEING AND COSMOS' INTEGRITY

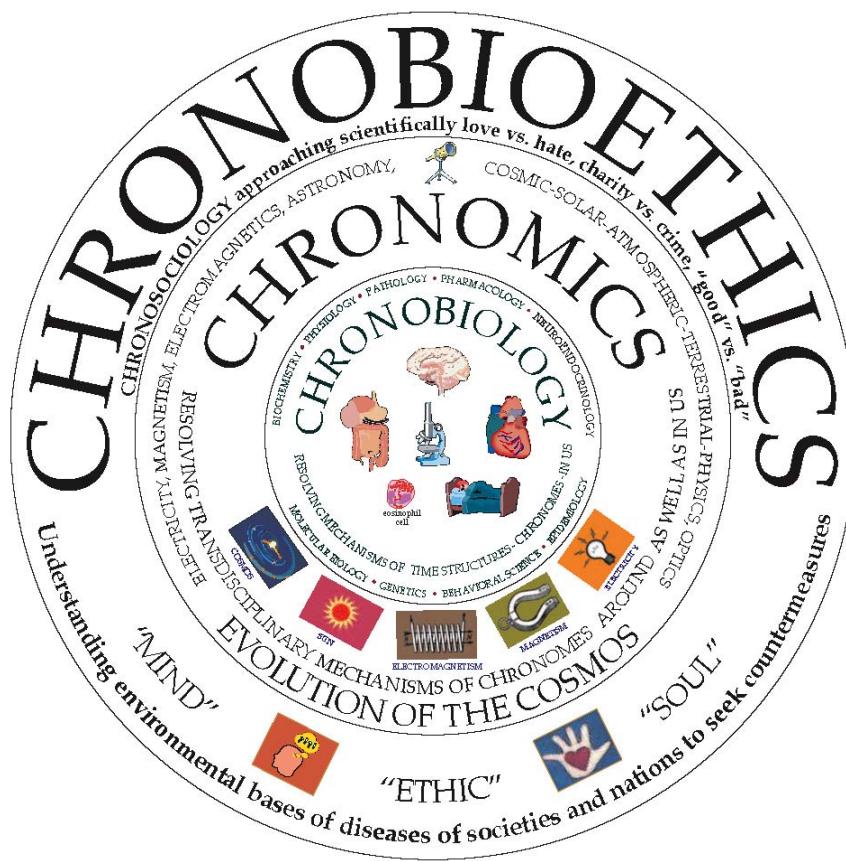


Figure 4. Chronobiology (microscopy in time, inner circle) uncovered mechanisms underlying the ubiquity, importance, and genetics of circadians and their synchronization with daily routines (3, 43). Chronomics (telescopy in time) reveals many other wobbly cycles not only in us, but also cycles around us, uncovering rhythms in the "good" and "bad", in religious motivation vs. crime and war. Monitoring blood pressure and heart rate as a start and mental functions, among others, is advocated, to start with for stroke prevention, but with the added aim of using the same approach for a rhythmometry focused on societal disease. Most recent findings reveal matching transyearly cycles of ~1.3 year length and other nonphotics in terrorism, heliogeomagnetics, systolic blood pressure, heart rate, and the self-rating of mood. Moreover, when a given spectral band in the satellite-measured speed of the solar wind or in terrestrial magnetism is (naturally) amplified or damped (by the sun), so are corresponding spectral bands in the incidence of terrorism in the last four decades (49, 50), and so is human 1-minute estimation. Removal of a broad transyearly spectral band in the solar wind's speed is accompanied by the partial loss and damping of the remaining corresponding band in the systolic blood pressure of an elderly man (12). The World Heart Journal could broaden the perspective (from "flying blind" between office visits or from a circadian bitemporal hemianopsia) to the spectrum in Figure 6 that shows many corresponding periods in and around us. Some of these cycles are associated with dying suddenly (12, 52) or by one's own hand (51) or being killed (49, 50). Chronobioethics serves as a basis for eventually developing timely and timed countermeasures to diseases not only of individuals but also of societies and civilizations (1-3), all within the broader scope of a new journal. © Halberg.

Prof. Singh's endeavors along chronobiologic lines are not new. He follows the late Frederic C. Bartter of Bartter syndrome fame, head of the then Hypertension and Endocrine Branch at the U.S. National Institutes of Health (NIH). As to BP, Bartter, who eventually became chief of the clinical center at NIH, wrote, about a patient whose BP was diagnosed differently by two other physicians who saw him at different times of day (19): "By conventional standards, this patient is clearly normotensive every morning. But the blood pressure determined each day at 6 in the afternoon provides especially convincing evidence that this patient is a hypertensive. ... My plea today [in 1974!] is that information contained in [data curves compiled under differing circumstances, such as 24 hours a day/7 days a week] become a *routine minimal amount* of information accepted for the description of a patient's blood pressure. *The analysis of this information by cosinor should become a routine.* It is essential that enough information be collected to allow objective characterization of a periodic phenomenon, to wit, an estimate of M [the time structure or chronome-adjusted mean, or MESOR] ... an estimate of [the amplitude] A itself, and finally an estimate of acrophase,  $\phi$  [a measure of timing]. In this way, a patient can be compared with himself at another time, or under another treatment, and the patient can be compared with a normal or with another patient" (19). Bartter then introduced the chronotherapy of hypertension (20), but his suggestion regarding diagnosis is also not new. Rather, it is in keeping with the insights of Theodore C. Janeway of Johns Hopkins Medical School, an opinion leader from the turn of the 20<sup>th</sup> century, who in 1904 wrote: "... *it is essential* that a record of the pressure be made at frequent intervals *at some time previous* [presumably to an examination], to establish the *normal level* and the *extent of the periodic variations*. When this is done, it may be possible to demonstrate changes of small extent, which, lacking this standard for comparison, would be considered within the limits of normal variation" (21).

Prof. Singh goes several important steps further, beyond spotchecks at **the** (putative "true", yet only instantaneous and fictitious if extrapolated beyond the moment of measurement) BP. He assesses the variability of this **variable** (sic). Instead of reference values applicable to everybody above 18 years, male

or female (17), he uses reference standards specified by age and gender. As compared to Janeway, who looked for periodicity with his unaided eyes, Prof. Singh uses time microscopy, with special computer methodology (22, 23), including the cosinor procedure that Bartter had recommended (19). Prof. Singh now collects reference standards specified for a broader than circadian time structure, realizing that to assess circadians, as some do on the basis of 24- or 48-hour records is equivalent to taking the pulse for one or two cycles (seconds!) (24-26).

Activities as a clinician, educator, epidemiologist and nutritionist, with basic contributions to the role of magnesium in heart health, were augmented when Prof. Singh founded and presided over the International College of Cardiology, with repeated, successful meetings (27, 28). Should a spotcheck "evidence"-based health care be supplanted with an instrumented self-surveillance (by his impact in the footsteps of the late Bartter in the US and the late Seshachar and the late Ramalingaswami in India), credit will go to Prof. R.B. Singh for many things (*multa*). Carl Friedrich Gauss, the titan of science, wanted much, but not many things (*multum sed non multa*). He wanted to be *intensive*, not *extensive*. Prof. Singh tries to do both. He brings much (chronobiologic tools and concepts) into the service of many things that are all pertinent to individual and international health and could thus lead to *multum*. To realize this goal, and by reference to a series of concerns discussed elsewhere (3), we suggest:

1. In the future, all authors in this new journal should be requested to provide data (whenever possible and pertinent) together with a manuscript before it is accepted for publication. Referees could thus see firsthand whether the conclusions stated in the paper are warranted. One of us heard in his student days that Justus von Liebig in the original Liebig's Annalen (29) in the 1830s did not publish a paper in his then prestigious journal before he did not successfully repeat the author's claim in his laboratory.
2. Mistakes as to new claims are an unpleasant fact and do occur. It is part of the referees' task to make sure that a paper is as sound as possible before it is accepted for publication.

Yet the best referees can err. Gauss, the king of mathematicians, erred in a judgement of a colleague's contribution in his own field. So will most of us who are shouldering more than we can carry. The refereeing process is sometimes problematic, insofar as it can be subjective, and some journals have adopted the policy to hide the authors' names to assure better objectivity in the review, a highly recommended procedure.

3. Mistakes are often committed in the choice of methodology used to analyze a given set of data. Some years ago, our laboratory reported substantial benefit from timing ara-C to treat leukemia in experimental animals (30-32), at variance with other researchers (33). The apparent discrepancy between the results of the two teams was readily resolved after it was found that the contenders (33) had not used the surviving (cured!) mice to calculate the average survival time, and thus ignored the merit of any cures (32)! The results were published in reputable journals (30-33). The contenders' results (33), to our knowledge, were never retracted.

The NIH, as does the profession at large, continue to disregard the benefit from chrono-chemotherapy or -radiotherapy (34-37). Radiation, when timed at the peak of the patient's tumor temperature, has doubled the 2-year disease free survival rate of patients with perioral cancers in India in the hands of Dr. B.D. Gupta and Dr. Akhil Deka, another Indian achievement (34, 38).

Chronobiology has also been largely ignored in cardiology, recommendations by several international consensus meetings (e.g., 39) based on the documented risks associated with abnormal dynamics within the normal range and the demonstrated benefits from chronotherapy notwithstanding. If the already-available information is disseminated, thanks to Prof. R.B. Singh, many strokes and other hard events may be prevented in his setting in Moradabad and in neighboring settings, as a model for the world as a whole, yet with modest local resources. We wish him success as chief editor in seeing to it that health care does not "fly blind", as another journal's leader put it in a spontaneous editorial (40).

## Epilogue

This editorial is dedicated to the memory of Kozo OKAMOTO, to whom we owe the spontaneously hypertensive rat (SHR), in particular the stroke-prone SHR (SHR-SP). Thanks to him, the diagnosis of essential or idiopathic hypertension, implying the absence of a known cause for high BP, can be scrutinized in its time course in the light of a model amenable to scrutiny in terms of its genetics and of the role played by environmental loads. In the pre-telemetry era, under the burdens of repeated 4-hourly measurements, with the animal in a wire mesh screen under a gooseneck lamp, circadian BP overswinging was first documented and led us to intensify focus on BP variability (41). Kozo Okamoto was well aware of the importance of doing this with due regard for rhythms: When a pharmaceutical company offered him a round trip ticket from Japan to the U.S. National Institutes of Health (NIH) (to which he was invited for lectures by a friend who was director of the NIH clinical center) and to the pharmaceutical company's headquarters, but the company would not pay for a side trip to Minnesota in their offering, Dr. Okamoto cancelled his visits to the NIH and to the pharmaceutical company, bought his own ticket to Minnesota, along with that of a colleague, and graced us with his and his colleague's presence as houseguests, and with the greatest gift, i.a., of the SHR-SP rats that led to the finding of circadian hyper-amplitude-tension under a load of handling for measurement by immobilization and heating for tail blood sphygmomanometry, but not in the absence of these acute burdens, albeit with a permanently implanted catheter (41). We have much to learn about the mechanisms involved in a worldwide hypertension and prehypertension (42-48), in the first model for prehypertension validated in humans. Telemetry is now available for rodents and, we trust, will someday become available for humans as well. Manual measurements can also serve (16, 42), if they are chronomically assessed. In advocating time-microscopy, the new World Heart Journal could open many eyes, just as in diagnosing a malignant tumor, we no longer rely on tumor, rubor, calor, and dolor, but use microscopy. By the same token, a microscopy in time is needed for hypertension and prehypertension not to remain silent to the care

receiver and care provider. A multilingual website, Figure 5 (<http://www.sphygmochron.org/>), now planned by the Phoenix Project of the Twin Cities chapter of the Institute of Electrical and Electronics Engineers (IEEE) (<http://www.phoenix.tc-ieee.org>), may serve for both self-help in health care and research, which has most recently revealed

associations of terrorism (49, 50) as well as suicides (51) and tachyarrhythmias (52) with heliogeomagnetics. Figure 6 broadens the perspective from circadian hemianopsia to a wide spectrum of novel components that relate to dying suddenly (52) or by one's own hand (51) or being killed (49, 50).

**Preventive (left) and curative (lower right) health care can yield the dividend of biomedical monitoring of space weather (top right) by time-structural analyses of ambulatory blood pressure and heart rate series<sup>1</sup>**

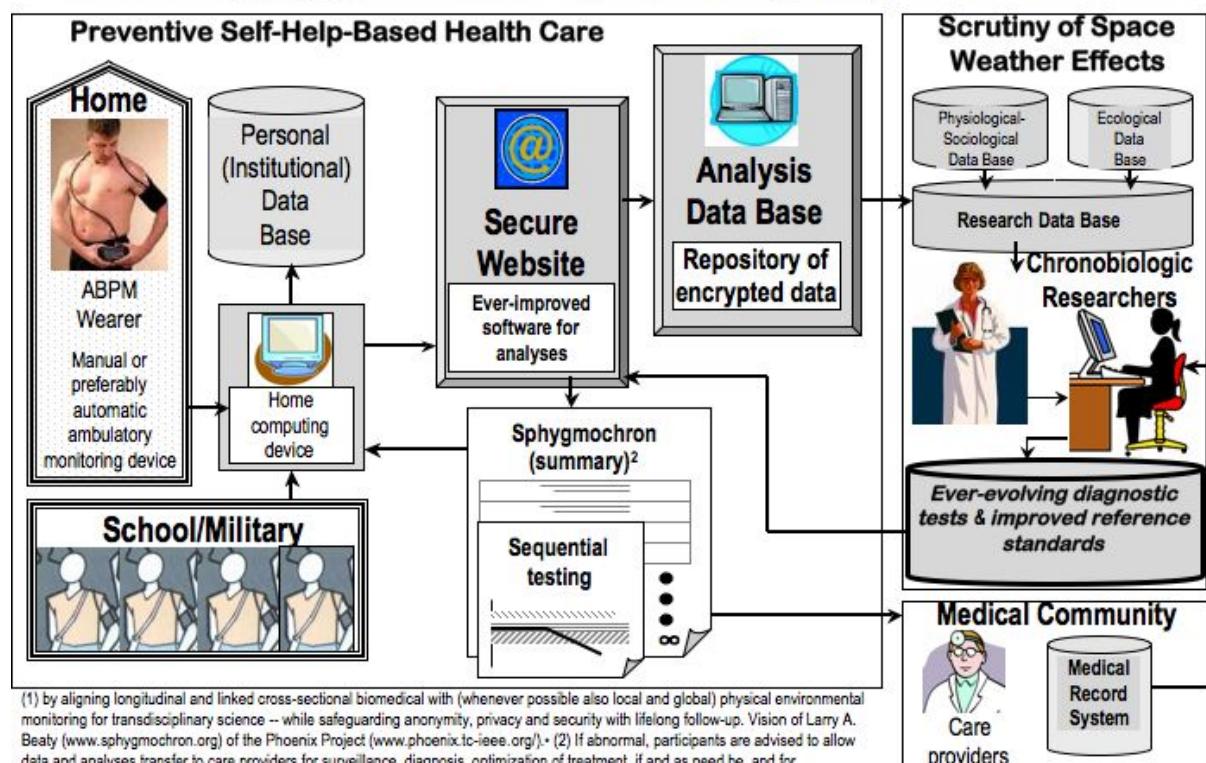
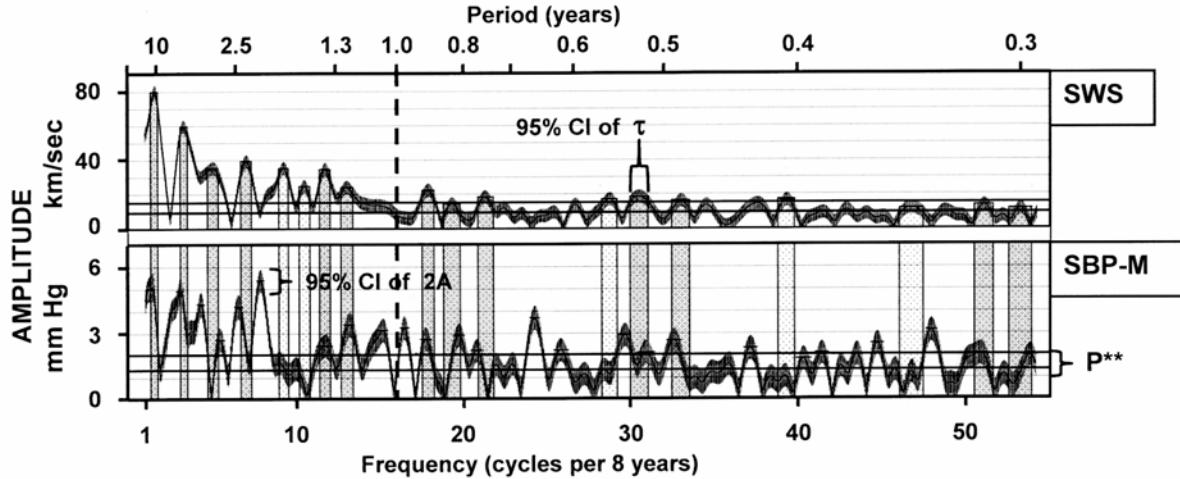


Figure 5. The Phoenix Project of volunteering members of the Twin Cities chapter of the Institute of Electrical and Electronics Engineers (<http://www.phoenix.tc-ieee.org>) is planning on developing an inexpensive, cuffless automatic monitor of blood pressure and on implementing the concept of a website ([www.sphygmochron.org](http://www.sphygmochron.org)) for a service in exchange for the data that in turn are to be used for refining methods and for monitoring psychophysiological effects of the variability in space weather. © Halberg.

**DECADAL, MULTI- & EXTRA-ANNUAL CONGRUENCE OR SIMILARITY  
GAUGED BY OVERLAPPING OR OVERLYING VS CONTIGUOUS CIs<sup>†</sup> OF PERIOD,  $\tau$ ,  
IN SPECTRA OF SOLAR WIND SPEED (SWS)  
AND SYSTOLIC BLOOD PRESSURE (SBP) MESOR (M) \***



\* SWS: daily data from [ftp://nssdcftp.gsfc.nasa.gov/spacecraft\\_data/omni](ftp://nssdcftp.gsfc.nasa.gov/spacecraft_data/omni), N=5272. BP data: daily averages from Dec 1989 to Jul 2006 monitored ~ every 30 min (with gaps) by FH, a man of 70 y at start of records. N = 2684.

\*\* Horizontal lines indicate ordering significance at the 0.001 and 0.05 levels only as first approximation; until more robust methods become available, that are not dependent upon the assumptions of independence, normality and homogeneity of variance, a transdisciplinary congruence of periods and a "remove - replace" approach remain the criteria of importance. Congruence between components with amplitudes differing from zero with an ordering P between 0.001 and 0.05 are questionable.

<sup>†</sup> CI = 95% confidence interval of  $\tau$  (of each component fitted separately).

Figure 6. Congruent periods,  $\tau$ , in the biosphere and around us (52) point to associations between the human circulation (as a gauge of biospheric response) and its cosmos (read: space weather). While no causal relations are implied by the findings as such of periods with overlapping if not overlying 95% confidence intervals, the subtraction and addition approach (12) provides evidence of interactions, awaiting associations with photic and nonphotic cycles on the one hand and morbidity and mortality cycles in solar activity on the other hand. Just as are the photic and thermal, now also societal cycles of the day and night and of the seasons, so may nonphotic cycles also be coded in our genes. © Halberg.

## References

- [1] Otsuka K, Cornélissen G, Schwartzkopff O, Bakken EE, Halberg F, Burioka N, Katinas GS, Kane R, Regal PJ, Schaffer E, Sonkowsky R, Patterson R, Engebretson M, Brockway B, Wang ZR, Delmore P, Halpin C, Sarkozy S, Wall D, Halberg J. Clinical chronobiology and chronome-geriatrics: At variance with recommendations of subsequent guidelines, yet focusing indeed on pre-hypertension in the physiological range. *Biomed Pharmacother* 2003; 57 (Suppl 1): 164s-198s.
- [2] Cornélissen G, Halberg F, Bakken EE, Singh RB, Otsuka K, Tomlinson B, Delcourt A, Toussaint G, Bathina S, Schwartzkopff O, Wang ZR, Tarquini R, Perfetto F, Pantaleoni GC, Jozsa R, Delmore PA, Nolley E. 100 or 30 years after Janeway or Bartter, Healthwatch helps avoid "flying blind". *Biomed Pharmacother* 2004; 58 (Suppl 1): S69-S86.
- [3] Halberg F, Cornélissen G, Katinas G, Tvidiani L, Gigolashvili M, Janashia K, Toba T, Revilla M, Regal P, Sothern RB, Wendt HW, Wang ZR, Zeman M, Jozsa R, Singh RB, Mitsutake G, Chibisov SM, Lee J, Holley D, Holte JE, Sonkowsky RP, Schwartzkopff O, Delmore P, Otsuka K, Bakken EE, Czaplicki J, International BIOCOS Group. Chronobiology's progress: Part II, chronomics for an immediately applicable biomedicine. *J Applied Biomedicine* 2006; 4: 73-86. [http://www.zsf.jcu.cz/vyzkum/jab/4\\_2/halberg2.pdf](http://www.zsf.jcu.cz/vyzkum/jab/4_2/halberg2.pdf).
- [4] Cornélissen G, Chen CH, Halberg F. Predictive value of blood pressure variability: merits of circadian parameters versus dipping patterns. *N Engl J Med* 2006 [Aug 14]; 355; 8: 850.
- [5] Halberg F, Cornélissen G, Halberg J, Schwartzkopff O. Pre-hypertensive and other variabilities also await treatment. *Am J Medicine* 2007; 120: e19-e20. doi:10.1016/j.amjmed.2006.02.045.
- [6] Cornélissen G, Halberg F, Otsuka K, Singh RB, Chen CH. Chronobiology predicts actual and proxy outcomes when dipping fails. *Hypertension* 2007; 49: 237-239. doi:10.1161/01.HYP.0000250392.51418.64.

- [7] Sanchez de la Pena S, Gonzalez C, Cornélissen G, Halberg F. Blood pressure (BP), heart rate (HR) and non-insulin-dependent diabetes mellitus (NIDDM) chronobiology. Abstract S8-06, 3rd Int Congress on Cardiovascular Disease, Taipei, Taiwan, 26-28 Nov 2004. *Int J Cardiol* 2004; 97 (Suppl 2): S14.
- [8] Halberg F, Cornélissen G, Sothern RB, Hillman D, Katinas GS, Nolley ES, Beaty LA, Otsuka K, Siegelova J, Greenway F, Gupta A, Revilla M, Masalov M, Syutkina EV, Malkova I, Chibisov SM, Schwartzkopff O, Bakken EE. Global challenges of monitoring vascular variability and space weather. In: Halberg F, Kenner T, Fiser B, Siegelova J, eds. *Proceedings, Noninvasive Methods in Cardiology* 2007, Brno, Czech Republic, November 11-14, 2007. Brno: Department of Functional Diagnostics and Rehabilitation, Faculty of Medicine, Masaryk University (ISBN 978 80 7018 463 4); 2007. p. 10-27.
- [9] Halberg F, Cornélissen G, Otsuka K, Watanabe Y, Katinas GS, Burioka N, Delyukov A, Gorgo Y, Zhao ZY, Weydahl A, Sothern RB, Siegelova J, Fiser B, Dusek J, Syutkina EV, Perfetto F, Tarquini R, Singh RB, Rhee B, Lofstrom D, Lofstrom P, Johnson PWC, Schwartzkopff O, International BIOCOS Study Group. Cross-spectrally coherent ~10.5- and 21-year biological and physical cycles, magnetic storms and myocardial infarctions. *Neuroendocrinol Lett* 2000; 21: 233-258.
- [10] Cornélissen G, Halberg F, Breus T, Syutkina EV, Baevsky R, Weydahl A, Watanabe Y, Otsuka K, Siegelova J, Fiser B, Bakken EE. Non-photic solar associations of heart rate variability and myocardial infarction. *J Atmos Solar-Terr Phys* 2002; 64: 707-720.
- [11] Halberg F, Cornélissen G, Regal P, Otsuka K, Wang ZR, Katinas GS, Siegelova J, Homolka P, Prikryl P, Chibisov SM, Holley DC, Wendt HW, Bingham C, Palm SL, Sonkowsky RP, Sothern RB, Pales E, Mikulecky M, Tarquini R, Perfetto F, Salti R, Maggioni C, Jozsa R, Konradov AA, Kharlitskaya EV, Revilla M, Wan CM, Herold M, Syutkina EV, Masalov AV, Faraone P, Singh RB, Singh RK, Kumar A, Singh R, Sundaram S, Sarabandi T, Pantaleoni GC, Watanabe Y, Kumagai Y, Gubin D, Uezono K, Olah A, Borer K, Kanabrocki EA, Bathina S, Haus E, Hillman D, Schwartzkopff O, Bakken EE, Zeman M. Chronoastrobiology: proposal, nine conferences, heliogeomagnetics, transyears, near-weeks, near-decades, phylogenetic and ontogenetic memories. *Biomed Pharmacother* 2004; 58 (Suppl 1): S150-S187.
- [12] Halberg F, Cornélissen G, Katinas G, Tvidiani L, Gigolashvili M, Janashia K, Toba T, Revilla M, Regal P, Sothern RB, Wendt HW, Wang ZR, Zeman M, Jozsa R, Singh RB, Mitsutake G, Chibisov SM, Lee J, Holley D, Holte JE, Sonkowsky RP, Schwartzkopff O, Delmore P, Otsuka K, Bakken EE, Czaplicki J, International BIOCOS Group. Chronobiology's progress: Part I, season's appreciations 2004-2005. Time-, frequency-, phase-, variable-, individual-, age- and site-specific chronomics. *J Applied Biomedicine* 2006; 4: 1-38. [http://www.zsf.jcu.cz/vyzkum/jab/4\\_1/halberg.pdf](http://www.zsf.jcu.cz/vyzkum/jab/4_1/halberg.pdf).
- [13] Cornélissen G, Halberg F, Singh RB, International BIOCOS Group. Editorial: unseen space weather also relates to cardiac events. This volume.
- [14] Nozawa M, Sugimoto K-i, Ohmori M, Ando H, Fujimura A. Dosing time-dependent effect of temocapril on the mortality of stroke-prone spontaneously hypertensive rats. *J Pharmacol Exp Ther* 2006; 316: 176-181.
- [15] Singh RB, Cornélissen G, Kumar A, Bathina S, Halberg F. Active prayer lowering heart rate variability along the scale of 168 hours in Asian Indians. *Proceedings, International Conference on the Frontiers of Biomedical Science: Chronobiology*, Chengdu, China, September 24-26, 2006, p. 193-195.
- [16] Singh RB, Cornélissen G, Siegelova J, Homolka P, Halberg F. About half-weekly (circasemiseptan) pattern of blood pressure and heart rate in men and women of India. *Scripta medica (Brno)* 2002; 75: 125-128.
- [17] Hagen P (ed.) *Mayo Clinic Guide to Self-Care: Answers for Everyday Health Problems*. Rochester, MN / Jacksonville, FL / Scottsdale, AZ: Mayo Clinic; 2003. p. 180-181.
- [18] Pickering TG, Shimbo D, Haas D. Ambulatory blood pressure monitoring. *N Engl J Med* 2006; 354: 2368-2374.
- [19] Bartter FC. Periodicity and medicine. In: Scheving LE, Halberg F, Pauly JE, eds. *Chronobiology*. Tokyo: Igaku Shoin Ltd., 1974: 6-13.
- [20] Güllner HG, Bartter FC, Halberg F. Timing antihypertensive medication. *The Lancet*, September 8, 1979: 527.
- [21] Janeway TC. *The clinical study of blood pressure*. New York: D. Appleton & Co., 1904, 300 pp.
- [22] Halberg F. Chronobiology. *Annu Rev Physiol* 1969; 31: 675-725.
- [23] Cornélissen G, Halberg F. Chronomedicine. In: Armitage P, Colton T, editors. *Encyclopedia of Biostatistics*, 2nd ed. Chichester, UK: John Wiley & Sons Ltd; 2005. p. 796-812.
- [24] Cornélissen G, Bakken E, Delmore P, Orth-Gomer K, Akerstedt T, Carandente O, Carandente F, Halberg F. From various kinds of heart rate variability to chronocardiology. *Am J Cardiol* 1990; 66: 863-868.
- [25] Wood MA, Simpson PM, London WB, Stambler BS, Herre JM, Bernstein RC, Ellenbogen KA. Circadian pattern of ventricular tachyarrhythmias in patients with implantable cardioverter-defibrillators. *J Am Coll Cardiol* 1995; 25: 901-907.
- [26] Halberg F, Cornélissen G, Otsuka K, Watanabe Y, Wood MA, Lambert CR, Zaslavskaya R, Gubin D, Petukhova EY, Delmore P, Bakken E. Rewards in practice from recycling heart rate, ectopy, ischemia, and

- blood pressure information. *J Medical Engineering & Technology* 1997; 21: 174-184.
- [27] Mitro P, Pella D, Rybar R, Valocik G, editors. *Proceedings, 2nd Congress on Cardiovascular Diseases*, Kosice, Slovakia, 25-27 April 2002. Bologna: Monduzzi Editore; 2002.
- [28] Abstracts of the 3<sup>rd</sup> International Congress on Cardiovascular Disease, Taipei, Taiwan, 26-28 Nov 2004. *Int J Cardiol* 2004; 97 (Suppl 2): S1-S81.
- [29] Liebigs Annalen/Recueil: Aims and Scope. [\(c\) 2003.](http://www.interscience.wiley.com/jpages/0947-3440/aims.html)
- [30] Haus E, Halberg F, Scheving L, Pauly JE, Cardoso S, Kuehl JFW, Sothern R, Shiotsuka RN, Hwang DS. Increased tolerance of leukemic mice to arabinosyl cytosine given on schedule adjusted to circadian system. *Science* 1972; 177: 80-82.
- [31] Halberg F, Haus E, Cardoso SS, Scheving LE, Kuehl 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.
- [32] Halberg F, Nelson W, Cornélissen G, Haus E, Scheving LE, Good RA. On methods for testing and achieving cancer chronotherapy. *Cancer Treatment Rep* 1979; 63: 1428-1430.
- [33] Rose WC, Trader MW, Lester WR Jr, Schabel FM Jr. Chronochemotherapy of L1210 leukemic mice with cytosine arabinoside or cyclophosphamide. *Cancer Treatment Reports* 1978; 62(9): 1337-1349.
- [34] Halberg F. Biological as well as physical parameters relate to radiology. Guest Lecture, *Proc. 30th Ann. Cong. Rad.*, January 1977, Post-Graduate Institute of Medical Education and Research, Chandigarh, India, 8 pp.
- [35] Halberg F, Gupta BD, Haus E, Halberg E, Deka AC, Nelson W, Sothern RB, Cornélissen G, Lee JK, Lakatua DJ, Scheving LE, Burns ER. Steps toward a cancer chronopolytherapy. In: *Proc. XIV International Congress of Therapeutics*. Montpellier, France: L'Expansion Scientifique Française; 1977. p. 151-196.
- [36] Halberg Francine, Halberg J, Halberg E, Halberg Franz. Chronobiology, radiobiology and steps toward the timing of cancer radiotherapy. In: Goldson AL (vol ed.) *Cancer Growth and Progression*, vol. 9, ch. 19, Kaiser H (series ed.) Dordrecht: Kluwer Academic Publ.; 1989. p. 227-253.
- [37] Cornélissen G, Halberg F. The chronobiologic pilot study with special reference to cancer research: Is chronobiology or, rather, its neglect wasteful? In: Goldson AL (vol ed.) *Cancer Growth and Progression*, vol. 9, ch. 9, Kaiser H (series ed.) Dordrecht: Kluwer Academic Publ.; 1989. p. 103-133.
- [38] Halberg F, Cornélissen G, Wang ZR, Wan C, Ulmer W, Katinas G, Singh Ranjana, Singh RK, Singh Rajesh, Gupta BD, Singh RB, Kumar A, Kanabrocki E, Sothern 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 nutriceuticals and beyond. *J Exp Therapeutics Oncol* 2003; 3: 223-260.
- [39] Halberg F, Cornélissen G, International Womb-to-Tomb Chronome Initiative Group: Resolution from a meeting of the International Society for Research on Civilization Diseases and the Environment (New SIRMCE Confederation), Brussels, Belgium, March 17-18, 1995: Fairy tale or reality? *Medtronic Chronobiology Seminar* #8, April 1995, 12 pp. text, 18 figures. <http://www.msi.umn.edu/~halberg/>
- [40] Fossel M. Editor's Note [to Halberg F, Cornélissen G, Halberg J, Fink H, Chen C-H, Otsuka K, Watanabe Y, Kumagai Y, Syutkina EV, Kawasaki T, Uezono K, Zhao ZY, Schwartzkopff O. Circadian Hyper-Amplitude-Tension, CHAT: a disease risk syndrome of anti-aging medicine]. *J Anti-Aging Med* 1998; 1: 239.
- [41] Halberg J, Halberg E, Hayes DK, Smith RD, Halberg F, Delea CS, Danielson RS, Bartter FC. Schedule shifts, life quality and quantity modeled by murine blood pressure elevation and arthropod lifespan. *Int J Chronobiol* 1980; 7: 17-64.
- [42] Stinson SM, Cornélissen G, Scarpelli PT, Halberg F. Self-measurement and ambulatory monitoring of blood pressure: a subject's chronobiological perspective. *Biomed Pharmacother* 2002; 56 (Suppl 2): 333s-338s.
- [43] Halberg Franz, Cornélissen G, Katinas G, Syutkina EV, Sothern RB, Zaslavskaya R, Halberg Francine, Watanabe Y, Schwartzkopff O, Otsuka K, Tarquini R, Perfetto P, Siegelova J. Transdisciplinary unifying implications of circadian findings in the 1950s. *J Circadian Rhythms* 2003; 1: 2. 61 pp. [www.JCircadianRhythms.com/content/pdf/1740-3391/1/2.pdf](http://www.JCircadianRhythms.com/content/pdf/1740-3391/1/2.pdf)
- [44] Cornélissen G, Delcourt A, Toussaint G, Otsuka K, Watanabe Y, Siegelova J, Fiser B, Dusek J, Homolka P, Singh RB, Kumar A, Singh RK, Sanchez S, Gonzalez C, Holley D, Sundaram B, Zhao Z, Tomlinson B, Fok B, Zeman M, Dulkova K, Halberg F. Opportunity of detecting pre-hypertension: worldwide data on blood pressure overswinging. *Biomed Pharmacother* 2005; 59 (Suppl 1): S152-S157.
- [45] Kumagai Y, Shiga T, Sunaga K, Cornélissen G, Ebihara A, Halberg F. Usefulness of circadian amplitude of blood pressure in predicting hypertensive cardiac involvement. *Chronobiologia* 1992; 19: 43-58.
- [46] Watanabe Y, Cornélissen G, Halberg F, Bingham C, Siegelova J, Otsuka K, Kikuchi T. Incidence pattern and treatment of a clinical entity, overswinging or circadian hyperamplitudetension (CHAT). *Scripta medica (Brno)* 1997; 70: 245-261.

- [47] Kumagai Y, Cornélissen G, Halberg F, Watanabe Y, Otsuka K, Schwartzkopff O, Singh RB. Kumagai's, Cugini's and other vascular variability disorders, detected chronobiologically, misdiagnosed by dipping. PS-050, *Proceedings, 2nd World Congress of Chronobiology*, November 4-6, 2007, Tokyo, Japan, p. 86.
- [48] Cornélissen G, Cugini P, Siegelova J, Fiser B, Halberg F. Cugini's minimal change hypertensive retinopathy, resolved chronobiologically while dipping fails, supports the concept of 'pre-hypertension'. In: Halberg F, Kenner T, Fiser B, Siegelova J, eds. *Proceedings, Noninvasive Methods in Cardiology 2007*, Brno, Czech Republic, November 11-14, 2007. Brno: Department of Functional Diagnostics and Rehabilitation, Faculty of Medicine, Masaryk University (ISBN 978 80 7018 463 4); 2007. p. 55-61.
- [49] Halberg F, Cornélissen G, Sothern RB, Chibisov SM, Wendt H. Do unseen, very weak magnetic mechanisms contribute to terrorism in wobbly spectral windows? *8th Int Cong "Health and education millennium"*, People's Friendship University of Russia, Moscow, Russia, November 14-17, 2007, in press.
- [50] Cornélissen G, Halberg F, Wendt H, Chibisov SM. Human violence-terrorism characterized by Aeolian weak magnetoperiodism, an ~1.3-year (transyear) cycle, rather than an annual socio-photo-thermoperiodism. *8th Int Cong "Health and education millennium"*, People's Friendship University of Russia, Moscow, Russia, November 14-17, 2007, in press.
- [51] Cornélissen G, Halberg F. Chronomics of suicides and the solar wind. *Br J Psychiatry* 2006; 189: 567-568.
- [52] Cornélissen G, Benser M, Halberg F. Patterns of incidence of tachyarrhythmias recorded in implantable cardioverter-defibrillators during 2001-2005. PS-004, *Proceedings, 2nd World Congress of Chronobiology*, November 4-6, 2007, Tokyo, Japan, p. 63.





## EDITORIAL

# Unseen Space Weather Also Relates to Cardiac Events

**Germaine Cornélissen\*, Franz Halberg\*, R.B. Singh• and the international BIOCOS (The Biosphere and the Cosmos) project<sup>1</sup>**

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Relationships between coronary disease and meteorology were documented in 10-year data as a 13% increase in event rates of all age groups ( $P<0.0001$ ) (18% for the 55- to 64-year olds) with a 10°C decrease in temperature (1), a finding in keeping with the highest fatality from acute myocardial infarction (MI) on the coldest days reported from Helsinki in 1977 (2) and other meteorological analyses in relation to MI (3). The effect of cold weather is also documented in the laboratory on days when ECG was monitored after ligation of the descending coronary artery in dogs: more sudden deaths occurred in November-February (42%) vs. July-August (6%) (4). Contradictory reports from subtropical climates claim that the seasons do not (5) or do (6) affect the frequency of acute MI. In cold cities, both high and low temperatures were associated with increased cardiovascular mortality (7).

As to hot temperatures acting specifically upon hospital admissions for heart disease, much of the effect has been interpreted as an increase with average temperature constituting a short-term displacement of pattern (8). Other preliminary data also suggest that atrial fibrillation-related mortality extends beyond colder climates to hotter climates (with sufficiently large relative changes in ambient temperature during the year) (9). This finding is in keeping with the number of deaths in patients with congestive heart failure after prior MI in cold- and warm-weather months being higher than that in spring and fall months (10).

Against this background, Pella et al. (11), based on a paper by the chief editor (12), review the role of hot, not only of cold climate in the genesis of MI. Apart from focus only upon the extremes of weather on earth triggering cardiac events, even the usual change of the seasons corresponding to the calendar year suffices to show, among other temporal factors, an association with MI, as seen in the middle of Figure 1 for Minnesota, USA (13). By comparison to the role of the photic and thermic and now also societal changes in the earth's proximate climate,

any association of MI with unseen space weather seems relatively small, although it is present, as shown in part by a circadecadal component (Figure 1, left) (13, 14). A far-transyear (15, 16) with a period of about 1.23 years and a CI (95% confidence interval) from 1.20 to 1.26 years and a near-transyear (17) of about 1.05 years with a CI not overlapping the precise calendar year are also present in MI in Minnesota (1968-1996). The far-transyear's amplitude is just 17% of that of the calendar year, Figure 2. The 10.7-year amplitude is 95% of that of the half-year, which

also constitutes a geomagnetic signature (18). In keeping with Pella et al. (11) and Singh et al. (12) and others (1-4, 6-10), however, photic and thermic components predominate in the spectrum of MI. Non-photics include a heliomagnetic circadecadal, a transyearly and a cis-half-yearly component (19, 20; cf. 21), all having a smaller amplitude. The half-year is also prominent, whether it accounts for a nonsinusoidal circannual component, or whether it represents in part a non-photonic geomagnetic effect (18).

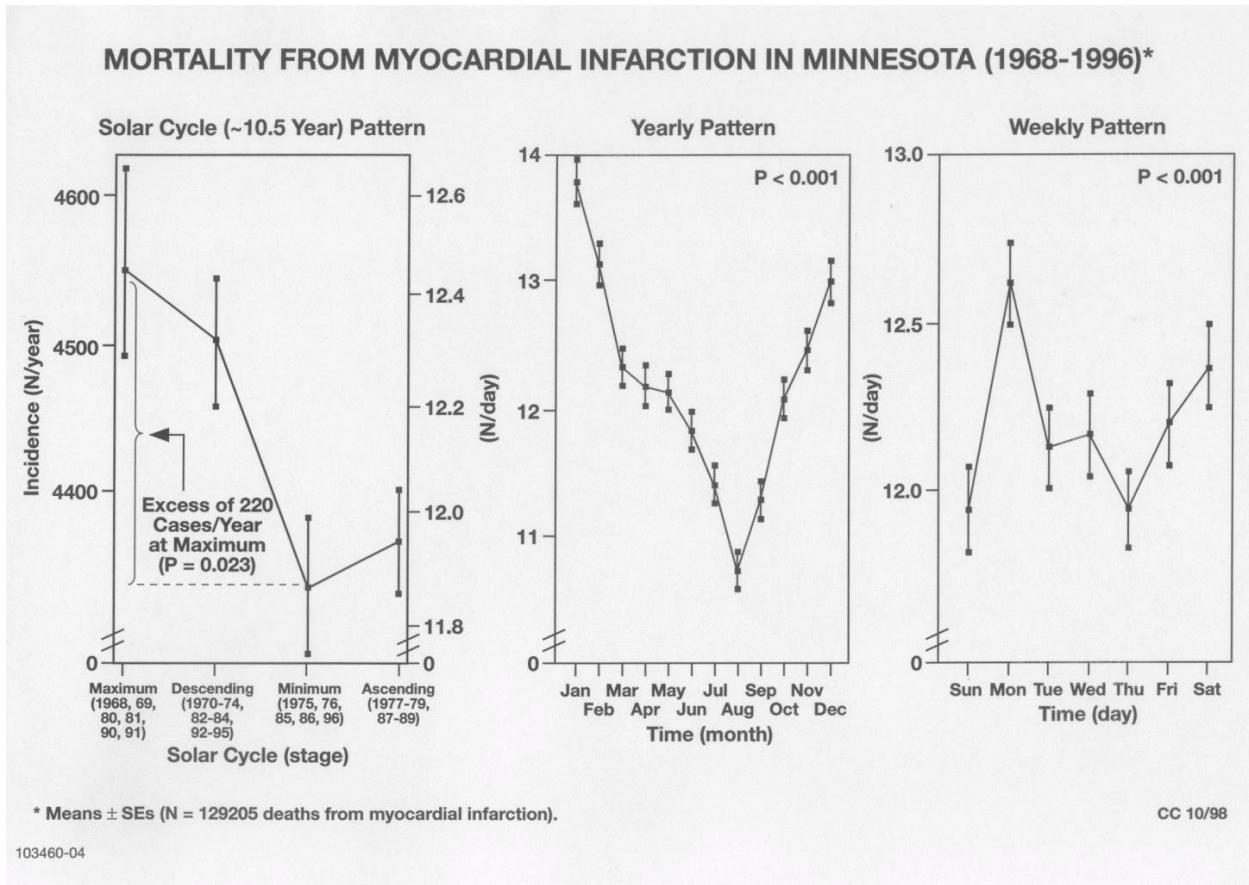


Figure 1. Non-random patterns of incidence of 129,205 deaths from myocardial infarction in Minnesota (1968-1996) along the scales of the photic and thermic year (middle), of the non-photonic helio- and geomagnetic about 6.75-day but socially synchronized week (right), and of Schwabe's about 10.5-year sunspot activity cycle (left). Data shown as means  $\pm$  SEs. As compared to times of solar minimum, there is an excess of 220 deaths/year at times of solar maximum (13,14). © Halberg.

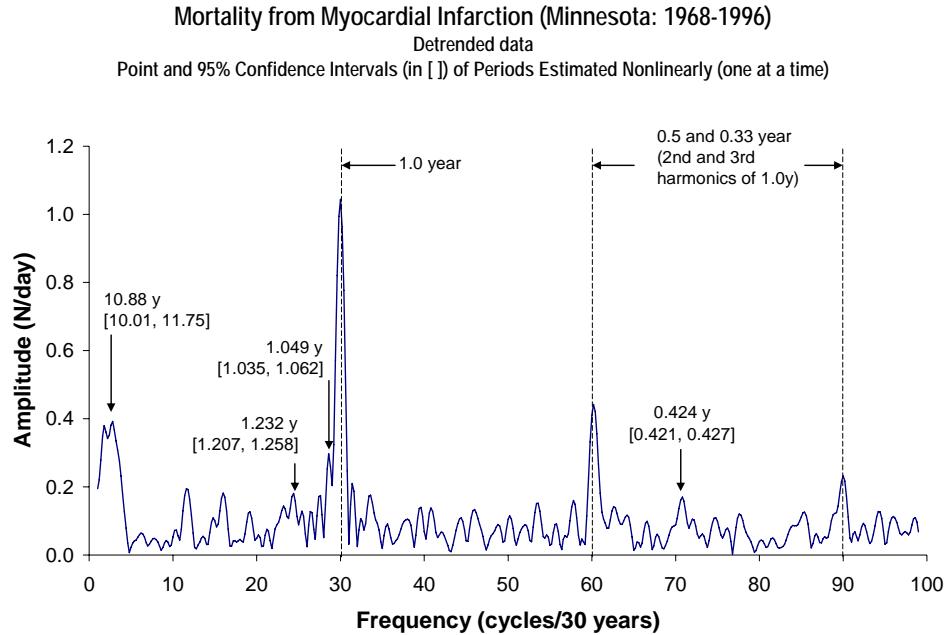


Figure 2. Spectral visualization of the importance of the photic circannual feature in mortality from myocardial infarction (vertical dashed lines and horizontal arrows at first 3 harmonics of 1 year). The vertical arrows emphasize separate peaks of probable non-photonic origin, with amplitudes much smaller than that of the photic yearly component. A near-transyear of about 1.05 years (in biology, beyond MI) is also found in other variables and has a 95% confidence interval (CI) not overlapping precisely one year. So does a far-transyear of about 1.23 years, corresponding roughly to a component often found in solar wind speed. The about 0.42-year cis-halfyear has a CI not overlapping the precise half-year, which could represent the waveform of the yearly component or could also be contributed by geomagnetics (18). By contrast, the cis-halfyear corresponds to a periodicity in hard solar flares (19, 20; cf. 21). © Halberg.

With the separation by the 10<sup>th</sup> revision of the International Classification of Diseases (ICD10) of MI from sudden cardiac death (SCD), space weather gains in importance in certain geographic, geomagnetic or dip-magnetic location. By contrast to MI, the latter electrical incidents of the heart (ICD10 code I46.1), at least in some specific localities and at certain times thus far (and not overall) have patterns that correspond to features of unseen space weather, Figure 3. A far-transyear, with a period longer than a year by a few months, characterizes the incidence of SCD in Arkansas, Minnesota, Tokyo (Japan), and the Czech Republic. A cis-half-year, with a period of about 5 months, is detected in Minnesota, Tokyo (Japan), Austria and the Czech Republic, among others. These spectral components resemble cycles seen in the speed of the ionized particles ejected by the sun, called the solar wind (22, 23), or in cosmic rays (24), and also found in geomagnetics (25). The prevalence in Minnesota solely of a transyear, longer than a calendar year by several months, with no calendar-yearly component in SCD contrasts with

both a calendar-year component and a transyear found in Arkansas, USA, and in the Czech Republic, and only a calendar-year component in North Carolina, USA, in Hungary, the Republic of Georgia, Latvia, Lithuania and Hong Kong, Figure 3 (21, 26-29). In addition to transyears, a signature of hard solar flares (19, 20) is also seen in SCD in Minnesota, Hungary and Lithuania, and part of the time in the Czech Republic (21). The contrast in Minnesota between SCD (Figure 3) and MI (Figure 2) awaits scrutiny on longer-than-available time series on both conditions in the same localities.

Clinical implications are:

1. The decision to separate MI from electrical accidents of the heart in ICD10 is supported in some geographic locations by drastically different patterns in spectra of their incidences, based on MI that exclude SCD vs. the spectra (of the non-photonic solar signatures) of SCD.

2. The signatures in SCD of transyears of the solar wind and cosmic rays and of cis-halfyears of hard solar flares are modulated by telluric factors that underlie the unequal geographic/geomagnetic/dip-magnetic distribution of non-photocyclic patterns and may provide clues to physical environmental influences associated with SCD.
3. To the extent that a weak correlation with geomagnetics of SCD (28) can be confirmed and the mechanisms of solar-terrestrial interaction in SCD are identified, improvements may be undertaken in devices such as cardiac pacemaker-cardioverter-defibrillators, notably since the incidence of tachyarrhythmia recorded by such devices may mimic the pattern of SCD (31).
4. The systematic recording of health statistics in different geographic locations (21, 30, cf. 32, 33) and their associations with environmental variables may help our understanding of underlying risks and may help design preventive countermeasures.
5. As the care recipient and caregiver learn about effects of space weather, a report on earth weather should gradually include also events occurring beyond our protective magnetosphere.

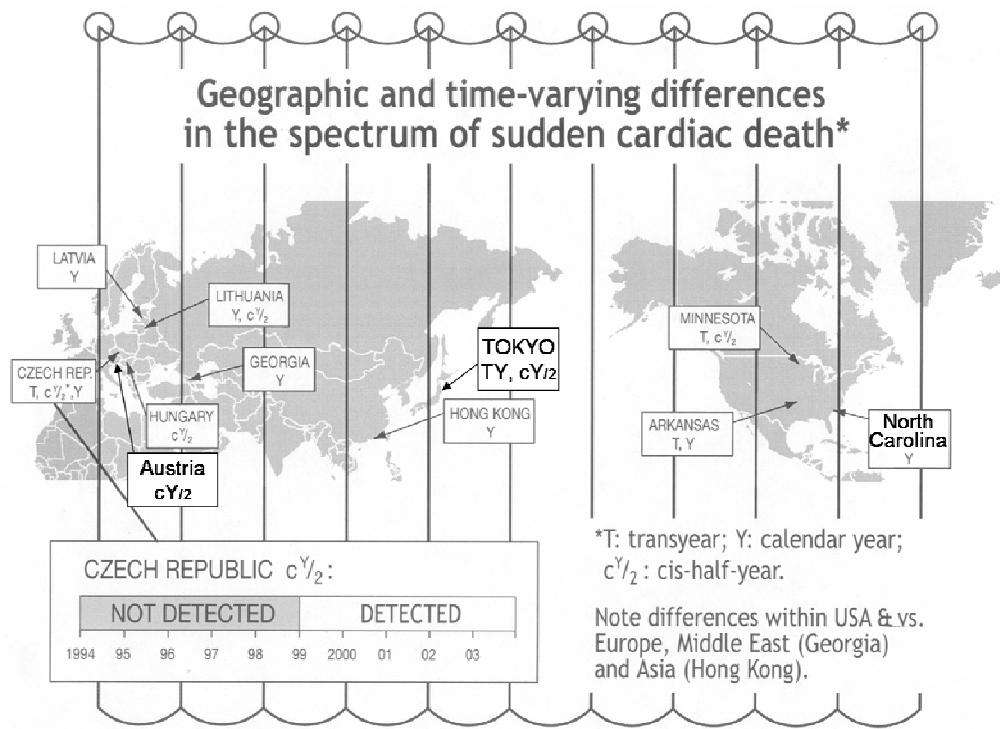


Figure 3. A curtain of uncertainty, because of limited available data, hides any time- and geographic (geomagnetic or dip-magnetic) site-specificity of various spectral aspects of sudden cardiac death (SCD). Thus, we find a transyear in Minnesota with a cis-half-year (cY/2) and both a calendar year and a transyear in Arkansas and the Czech Republic: at the latter site, a cis-half-year, corresponding to an also-transient cycle in hard solar flares (19, 20), is detected after but not before 1999 (21). In 1998, Minorsky (30) re-evaluated latitudinal differences in coconut palms' foliar spiral direction (FSD). He hypothesized that latitude-dependent biases in FSD, a non-Mendelian trait, will be associated with a temporally varying component of the earth's magnetic field. Earth currents, which are measurable in trees, bias the difference of auxin (or auxin transport proteins) in young embryos, such that left-handed palm trees (LHP) are produced preferentially in the Northern Hemisphere and right-handed ones (RHP) in the Southern Hemisphere. He built his observations on a previously collected data base by Davis and Davis, revealing that the ratio of LHP – RHP / total was better correlated with magnetic (dip) latitude than with geographic or geomagnetic (centered dipole) latitude. Minorsky and Bronstein (32) referred to this classical case of morphological antisymmetry in which dextral and sinistral forms are not inherited and are equally common within a species, proposing that P<sub>c1</sub>-induced earth currents may bias the diffusion of morphogens in coconut palm embryos, thereby giving rise in asymmetries of FSD. Whether other geographic differences in SCD may also relate to any magnetic latitude deserves scrutiny, notably since cardiac arrhythmias can also transiently reveal a transyear or a cishalfyear, each in a different solar Schwabe cycle stage (21). © Halberg.

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## References

- [1] Danet S, Richard F, Montaye M, Beauchant S, Lemaire B, Graux C, Cottel D, Marécaux N, Amouyal P. Unhealthy effects of atmospheric temperature and pressure on the occurrence of myocardial infarction and coronary deaths. A 10-year survey: the Lille-World Health Organization MONICA Project (Monitoring Trends and Determinants in Cardiovascular Disease). *Circulation* 1999; 100: e1-e7.
- [2] Sarna S, Romo M, Siltanen P. Myocardial infarction and weather. *Annals of Clinical Research* 1977; 9: 222-232.
- [3] Cohen JC, der Megreditchian G, Gerbier N, Choisnel E, Pezzi-Giraud D, Pasteyer J, Poisvert M, Besancon F. Prévision des recrudescences d'infarctus du myocarde, fondée sur une analyse météorologique multivariée. *Semaine des Hôpitaux* 1984; 60 (9): 598-601.
- [4] Scherlag BJ, Patterson E, Lazzara R. Seasonal variation in sudden cardiac death after experimental myocardial infarction. *J Electrocardiology* 1990; 23: 223-230.
- [5] Ku CS, Yang CY, Lee WJ, Chiang HT, Liu CP, Lin SL. Absence of a seasonal variation in myocardial infarction onset in a region without temperature extremes. *Cardiology* 1998; 89: 277-282.
- [6] Sharovsky R, Cesar LA, Ramires JA. Temperature, air pollution, and mortality from myocardial infarction in São Paulo, Brazil. *Brazilian J Med Biol Res* 2004; 37: 1651-1657.
- [7] Braga AL, Zanobetti A, Schwartz J. The effect of weather on respiratory and cardiovascular deaths in 12 U.S. cities. *Environmental Health Perspectives* 2002; 110: 859-863.
- [8] Schwartz J, Samet JM, Patz JA. Hospital admissions for heart disease: the effects of temperature and humidity. *Epidemiology* 2004; 15: 755-761.
- [9] Kiu A, Horowitz JD, Stewart S. Seasonal variation in AF-related admissions to a coronary care unit in a "hot"

- climate: fact or fiction? *J Cardiovascular Nursing* 2004; 19: 138-141.
- [10] Aronow WS, Ahn C. Elderly nursing home patients with congestive heart failure after myocardial infarction living in New York City have a higher prevalence of mortality in cold weather and warm weather months. *J Gerontology Series A: Biological Sciences & Medical Sciences* 2004; 59: 146-147.
- [11] Pella D, Dudova D, Kumar R, Gupta SB. Not only a cold climate but also a hot climate can trigger cardiac events. *World Heart J* 2007; 1: 129-130.:
- [12] Singh RB, Pella D, Sharma JP, Rastogi S, Kartikey K, Goel VK, Sharma R, Neki NS, Kumar A, Otsuka K. Increased concentrations of lipoprotein(a), circadian rhythms and metabolic reactions evoked by acute myocardial infarction associated with acute reactions in relation to large breakfasts. *Biomed Pharmacother* 2004; 58 (Suppl 1): S111-S115.
- [13] Cornélissen G, Halberg F, Schwartzkopff O, Delmore P, Katinas G, Hunter D, Tarquini B, Tarquini R, Perfetto F, Watanabe Y, Otsuka K. Chronomes, time structures, for chronobioengineering for "a full life". *Biomed Instrum Technol* 1999; 33: 152-187.
- [14] Cornélissen G, Halberg F, Breus T, Syutkina EV, Baevsky R, Weydahl A, Watanabe Y, Otsuka K, Siegelova J, Fiser B, Bakken EE. Non-photic solar associations of heart rate variability and myocardial infarction. *J Atmos Solar-Terr Phys* 2002; 64: 707-720.
- [15] Halberg F, Cornélissen G, Schack B, Wendt HW, Minne H, Sothern RB, Watanabe Y, Katinas G, Otsuka K, Bakken EE. Blood pressure self-surveillance for health also reflects 1.3-year Richardson solar wind variation: spin-off from chronomics. *Biomed Pharmacother* 2003; 57 (Suppl 1): 58s-76s.
- [16] Cornélissen G, Masalov A, Halberg F, Richardson JD, Katinas GS, Sothern RB, Watanabe Y, Syutkina EV, Wendt HW, Bakken EE, Romanov Y. Multiple resonances among time structures, chronomes, around and in us. Is an about 1.3-year periodicity in solar wind built into the human cardiovascular chronome? *Human Physiology* 2004; 30 (2): 86-92.
- [17] Halberg F, Cornélissen G, Katinas G, Sampson M, Schwartzkopff O, members of the BIOCOS project, Spector NH, Faraone P, Tomescu S, Hriscu M. In memoriam: Ion Baciu. Mutually supporting neartransyears in solar and terrestrial magnetics, microbial and cell biology, physiology and pathology. In: Cornélissen G, Kenner R, Fiser B, Siegelova J (eds.) *Proceedings, Symposium: Chronobiology in Medicine*. Dedicated to the 85<sup>th</sup> Anniversary of Professor Franz Halberg. Brno: Masaryk University; 2004. p. 78-86.
- [18] Cornélissen G, Halberg F, Pöllmann L, Pöllman B, Katinas GS, Minne H, Breus T, Sothern RB, Watanabe Y, Tarquini R, Perfetto F, Maggioni C, Wilson D, Gubin D, Otsuka K, Bakken EE. Circasemiannual chronomics: half-yearly biospheric changes in their own right and as a circannual waveform. *Biomed Pharmacother* 2003; 57 (Suppl 1): 45s-54s.
- [19] Rieger A, Share GH, Forrest DJ, Kanbach G, Reppin C, Chupp EL. A 154-day periodicity in the occurrence of hard solar flares? *Nature* 1984; 312: 623-625.
- [20] Ballester JL, Oliver R, Carbonell M. The near 160-day periodicity in the photospheric magnetic flux. *Astrophys J* 2002; 566: 505-511.
- [21] Halberg F, Cornélissen G, Katinas G, Tvidiani L, Gigolashvili M, Janashia K, Toba T, Revilla M, Regal P, Sothern RB, Wendt HW, Wang ZR, Zeman M, Jozsa R, Singh RB, Mitsutake G, Chibisov SM, Lee J, Holley D, Holte JE, Sonkowsky RP, Schwartzkopff O, Delmore P, Otsuka K, Bakken EE, Czaplicki J, International BIOCOS Group. Chronobiology's progress: Part I, season's appreciations 2004-2005. Time-, frequency-, phase-, variable-, individual-, age- and site-specific chronomics. *J Applied Biomedicine* 2006a; 4: 1-38. [http://www.zsf.jcu.cz/vyzkum/jab/4\\_1/halberg.pdf](http://www.zsf.jcu.cz/vyzkum/jab/4_1/halberg.pdf).
- [22] Richardson JD, Paularena KI, Belcher JW, Lazarus AJ. Solar wind oscillations with a 1.3-year period. *Geophys Res Lett* 1994; 21: 1559-1560.
- [23] Mursula K, Zieger B. The 1.3-year variation in solar wind speed and geomagnetic activity. *Adv Space Res* 2000; 25: 1939-1942.
- [24] Valdés-Galicia JF, Pérez-Enriquez R, Otaola JA. The cosmic-ray 1.68-year variation: a clue to understand the nature of the solar cycle? *Solar Physics* 1996; 167: 409-417.
- [25] Halberg F, Cornélissen G, Panksepp J, Otsuka K, Johnson D. Chronomics of autism and suicide. *Biomed Pharmacother* 2005; 59 (Suppl 1): S100-S108.
- [26] Halberg F, Cornélissen G, Schwartzkopff O, Bakken EE. Cycles in the biosphere in the service of solar-terrestrial physics? In: Schroeder W (ed.) *Case studies in physics and geophysics*. Bremen: Wilfried Schroeder/Science Edition, 2006, p. 39-87.
- [27] Halberg F, Cornélissen G, Gigolashvili M, Katinas G, Sothern RB, Schwartzkopff O, Otsuka K, Bakken EE. Chronomics and sudden cardiac death: a geographic challenge? In: *Proceedings, 59<sup>th</sup> Annual Meeting*, Japan Society of Neurovegetative Research, Tokyo, November 1-3, 2006, p. 33-36.
- [28] Cornélissen G, Halberg F, Rostagno C, Otsuka K. A chronomic approach to cardiac arrhythmia and sudden cardiac death. In: *Proceedings, 59<sup>th</sup> Annual Meeting*, Japan Society of Neurovegetative Research, Tokyo, November 1-3, 2006, p. 56-59.
- [29] Halberg F, Cornélissen G, Otsuka K, Fiser B, Mitsutake G, Wendt HW, Johnson P, Gigolashvili M, Breus T, Sonkowsky R, Chibisov SM, Katinas G, Siegelova J, Dusek J, Singh RB, Berri BL, Schwartzkopff O. Incidence of sudden cardiac death, myocardial infarction and far- and near-transyears. *Biomed Pharmacother* 2005; 59 (Suppl 1): S239-S261.

- [30] Minorsky PV. Latitudinal differences in coconut foliar spiral direction: a re-evaluation and hypothesis. *Annals of Botany* 1998; 82: 133-140.
- [31] Cornélissen G, Benser M, Halberg F. Patterns of incidence of tachyarrhythmias recorded in implantable cardioverter-defibrillators during 2001-2005. PS-004, *Proceedings, 2<sup>nd</sup> World Congress of Chronobiology*, November 4-6, 2007, Tokyo, Japan, p.63.
- [32] Minorsky PV, Bronstein NB. Do geomagnetic variations affect the foliar spiral direction of coconut palms? *Eos Trans. AGU* 2005; 86 (52), Fall Meeting Suppl., Abstract GP33A-0103.
- [33] Crawford VLS, Cornélissen G, Fiser B, Dusek J, Siegelova J, Halberg F. Infrannual changes in the incidence of myocardial infarctions and strokes in Northern Ireland. Abstract, *Noninvasive methods in cardiology*, 2.11.2004, Congress MEFA, Brno, Czech Republic, 2.-4.11.2004, p. 9.



## EDITORIAL

# Myocardial Viability Evaluation by Delayed Contrast MRI

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Ischemic injury causes loss of myocardial viability at injury sites. Revascularization intervention has become state of the art method of choice to heal up the evolving myocardial infarctions. With growing potentials and newer developments of the noninvasive imaging methods the attention was diverted to measure and identify the myocardial viability in subendocardial inferior wall and apex along with possibility of hibernating myocardium and revascularization benefits on LV function [1,2,3,4]. Myocardial viability quantitation depends on the success of various imaging methods such as delayed Magnetic Resonance Imaging (MRI), <sup>201</sup>Tl SPECT, Dobutamine stress echocardiography (DSE) in measurement of wall thickness, transmural extent with high predictive value of functional recovery after revascularization or other cardiac intervention. The recent trend indicates that evaluation of myocardial viability of dysfunctional myocardium by delayed contrast MRI has the potential to predict the functional recovery after revascularization in ischemic heart disease [5]. Scintigraphic techniques and DSE were routinely used to identify high-risk patients who could benefit from revascularization but inconclusive [5,6,7,8]. It is too early to say due to very limited data is available comparing cardiac MRI, <sup>201</sup>Tl SPECT and DSE. Moreover, there is paucity in information on the sensitivity, specificity, reproducibility, predictability, diagnostic accuracy, cost-effect reliability and precision of delayed contrast-enhanced cardiac MRI in comparison with <sup>201</sup>Tl SPECT and DSE to assess myocardial viability in predicting functional recovery after revascularization in patients with chronic ischemic heart failure. The current opinion is focused on the evaluation of myocardial viability by delayed contrast MRI with its advantages over other imaging <sup>201</sup>Tl SPECT and DSE techniques in prediction of functional recovery, efficacy of revascularization and limitations of existing imaging techniques. In this issue, Wu et al. have attempted remarkably to address these issues with novelty of delayed contrast MRI method in 40 chronic patients.

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In recent years, the value of cardiac MRI was highlighted to assess myocardial fibrosis and scar burden, coronary perfusion and contractile reserve with excellent image quality [9,10,11,12]. Different approaches using intravenous administration of a gadolinium-chelate (such as Gd-DTPA) were reported such as inversion-recovery prepared T<sub>1</sub>-weighted gradient-echo sequence, delayed enhancement to visualize regions of old infarction appearing nonviable by <sup>201</sup>Tl scintigraphy. High resolution MRI further improved the sensitivity for non-transmural infarction in evaluation of possible functional recovery after revascularization of dysfunctional myocardium [12, 13]. Recovery of regional and global LV function was reported unpredictable in the group of patients with residual MRI contrast [13, 14, 15]. However, the value of myocardial MRI in predicting functional recovery after revascularization in patients with chronic LV dysfunction, in comparison with well-established <sup>201</sup>Tl SPECT and DSE is lacking and remains to be established. MRI and DSE of tissue perfusion after coronary revascularization reflect the severity of microvascular disruption, occlusion, or extra-vascular compression by edema or hemorrhage. Thus, the revascularization patterns serve as surrogates of myocardial injury rather than a direct indicator of myocardial cellular viability [16,17,18]. A potential advantage of the MRI approach over other imaging methods is that MRI provides quantitative edge definition of the endocardial and epicardial surface for precise measurement of systolic wall thickening, end-diastolic wall thickness as morphological indicator of likely viability of an ischemically impaired region.

Application of delayed MRI techniques for myocardial viability in a region of ischemic injury can be grouped into 3 categories:

1. MRI evaluation of myocardial perfusion after revascularization,
2. comparison of MRI with <sup>201</sup>Tl SPECT and DSE response of myocardial contractile reserve, and
3. use of MRI contrast in morphological characterization of myocardial functional recovery.

The first MRI approach by Rogers et al. established the myocardial contrast enhancement on both first-pass and delayed MRI images[17]. The contrast enhancement identified that hypoenhancement on first-pass imaging was an indicator of poor functional recovery after the acute event. Hyperenhancement was observed in the periphery of the infarction or entire infarction in these patients. MRI also demonstrated the hypoenhancement regions within the infarct zone in patients with microvascular obstruction in the core of the infarction with severe LV remodeling[13,17]. It indicated that MRI hyperenhancement in the core of acute infarctions after revascularization either on first-pass or delayed MRI is predictive of more severe injury with possible poor functional recovery and poor outcome.

The second MRI approach may predict both myocardial viability and contractile reserve. It can measure the subendocardial wall thickness comparable with dobutamine echocardiography demonstrating residual contractile response in an ischemically injured or chronically ischemic region[19]. Quantitative assessment of dobutamine-induced systolic wall thickening on cine MRI serves as a reliable indicator of regional LV function improvement and ejection function after revascularization. <sup>19</sup>F-fluorodeoxyglucose PET for residual viability, dobutamine transesophageal echocardiography (TEE) and dobutamine cine MRI for prior myocardial infarction suggested the sensitivity and specificity of both dobutamine TEE and cine MRI for PET-defined viability [19]. An end-diastolic wall thickness measured by cine MRI in ischemic dysfunction segments appeared indicative of myocardial viability by <sup>201</sup>Tl SPECT radionuclide imaging or favorable response to revascularization.

A third MRI approach for determining myocardial viability in a region of ischemic injury uses dysprosium Dy-DTPA or GdDTPA as MRI contrast agents to probe cellular membrane integrity. Both of these contrast agents display extracellular distribution and are excluded by myocardial cell membranes. Dy-DTPA attenuates the intracellular signal due to its distribution in extracellular space and tissue microheterogeneity. A series of rapid inversion-recovery echo-planar images measured the precise *in vivo* measurement of T<sub>1</sub> relaxation constants of

myocardium using optimized inversion time at which T1 signal was nulled in both normal and ischemic myocardium on its pre-contrast and post-contrast MRI scans [20]. T1 relaxation constants of Gd MRI contrast media determined the distribution volume of Gd chelates in normal and ischemically injured myocardium. Contrast distribution volume may provide an index of the percentage of necrotic myocardial cells within a zone of ischemic injury [20]. The relative concentration of Gd chelate contrast media in myocardium and blood can be determined by its relative distribution volume in the myocardium. The distribution volume in ischemic injured myocardium may be observed slightly expanded by interstitial edema but significantly increased by loss of myocardial cellular membrane integrity. The distribution volume of MRI contrast media might be used to estimate the percentage of nonviable cells in a region of revascularized ischemic injury. At equilibration, the relative concentration of Gd DTPA-BMA in myocardium and blood can be determined by the relative distribution volume in the acute myocardial infarction<sup>1</sup>. During the past decade, high-molecular-weight MRI contrast agents were used to keep the contrast agents in the blood pool for several hours to improve coronary MRA [21]. Blood pool contrast media showed enhancement of the infarct periphery zone and demarcated a less enhanced central core in reperfused ischemic injury of graded severity [17,18,21]. It seems that delivery of the blood pool contrast agent to the core of the infarction depends on the duration of contrast media and intact microvasculature. However, low-molecular-size extracellular contrast agents can easily reach the infarction core by diffusion even in case of microvascular damage.

In recent years, the well known major problems were identified as *in vivo* assessment of myocardial viability, inferior heart wall, scars and evaluating their relationship with functional recovery after revascularization and contractile reserve to make the decision of myocardial intervention or heart transplantation. Delayed contrast enhancement on MRI and reduced uptake by <sup>201</sup>Tl SPECT suggested the efficacy of heart transplantation based on the myocardial viability index in percutaneous coronary intervention (PCI) and coronary bypass surgery (CABG) patients with or without scars and their

functional recovery after revascularization and restored contractile reserve by dobutamine response [1,7,14,20,21]. MRI and <sup>201</sup>Tl SPECT both may indicate new hyperenhancement segments in their post-revascularization scan in CABG and PCI groups to evaluate the LVEF, total LV mass, LV end-diastolic volume index, LV end-systolic volume index. As of today, the use of both MRI and <sup>201</sup>Tl SPECT methods is in infancy to use them as clinical The increased subendocardial wall thickening, at inferior apex and systolic wall thickening determined by MRI may not be conclusive to show functional recovery but depends on its transmural scar extent. The delayed MRI displayed better diagnostic accuracy, sensitivity, specificity, positive predictive value and negative predictive value of viability over <sup>201</sup>Tl SPECT. Myocardial contrast echocardiography determines myocardial reperfusion in acutely occluded epicardial coronary arteries [22]. Myocardial viability assessment by <sup>18</sup>F-FDG PET is limited due to its high cost.

In the current view, delayed MRI contrast enhancement analysis may be considered better than reduced radionuclide uptake <sup>201</sup>Tl SPECT or DSE response. However, contractile reserve detection by DSE and myocardial viability with scar detection in the proportion of transmural extent of delayed enhancement on MRI may provide better clue but observations are inconclusive. Chronic CAD patients with LV dysfunction undergoing revascularization indicated the functional recovery with both the extent and severity of nonviable tissue by MRI due to relationship of chronic CAD and severe LV dysfunction [22,23]. Contractile reserve may be observed as impaired with no scar or minor scar detected on MRI. Extensive scar on MRI and/or SPECT can have contractile function at rest. A clear advantage of MRI over <sup>201</sup>Tl SPECT is its better capability to distinguish the myocardial recovered function with viable myocardium from the visible scar without recovery after revascularization. In a recent study, MRI identified the subendocardial infarcts but missed by <sup>201</sup>Tl SPECT [22,23]. <sup>201</sup>Tl SPECT might overestimate the scar tissue, especially in the inferior wall and apex. The inferior wall is one of the most severely attenuated regions in the heart. So, greater attenuation of <sup>201</sup>Tl in the inferior segments is commonly observed in <sup>201</sup>Tl SPECT. Another

limitation of dysfunctional myocardium viable on  $^{201}\text{TL}$  SPECT and not on MRI can be due to the presence of a thin rim of viable tissue. Myocardial viability detected by MRI may be valuable on the clinical evaluation of potential benefits from high-risk revascularization procedures. However, several factors should be considered affecting the functional recovery such as intervention procedures, completeness of revascularization, and perioperative myocardial infarctions [24]. Other limitations in the evaluation of myocardial viability may be small and limited patients with revascularization receiving simultaneous DSE and  $^{201}\text{TL}$  imaging or receiving no revascularization at all. So, it complicates the MRI prediction of functional recovery.

In consideration of the time-series evaluation of myocardial viability, delayed contrast MRI and  $^{201}\text{TL}$  SPECT are reported in this issue as a possible index of potential myocardial viability in patients with revascularized myocardial infarctions. Using delayed MRI, the first-pass distribution of MRI contrast media indicated that the reperfusion at the myocardium tissue level was impeded in more than half of the injured regions. The cause of impeded perfusion was predictive of poorer contractile recovery 7 weeks after the acute event. This study demonstrated good correlations among DSE,  $^{201}\text{TL}$  SPECT and MRI in the detection of myocardial viability. These observations as mentioned in the paper by Wu et al. corroborated with earlier reports [25,26,27].

In conclusion, use of MRI for the evaluation of ischemic heart disease was proposed two decades ago. However,  $^{201}\text{TL}$  SPECT radionuclide imaging and echocardiography DSE have been considered useful in diagnosis of ischemic heart disease due to easy accessibility. Recently, non-invasive MRI imaging modality has emerged as cost-effective promising technique with lot of new imaging options in ischemic heart disease in prediction of myocardial viability after ischemic injury. The present view on myocardial viability and functional recovery evaluation by using radionuclide imaging, MRI and DSE will certainly open new opportunities of predicting the viability index and limitations of each technique in morphological, interventional assessment of ischemic heart disease in clinical decision making. The success of MRI multiple capabilities in ischemic heart disease will also depend on robust and fast techniques

developed for coronary artery MRA angiographic methods. Presently, the coupled noninvasive coronary MRA with flow measurement appears as method of choice in the diagnosis of coronary artery disease. Cardiovascular MRI may provide a unique tool to assess cardiac structure and function in a single study session. The correlation among DSE,  $^{201}\text{TL}$  SPECT and MRI in the detection of myocardial viability, MRI detects viability better than  $^{201}\text{TL}$  SPECT, especially in the inferior wall and apex. Cardiac MRI certainly has higher predictive value of predicting functional recovery. In severe ischemic cardiomyopathy, MRI may provide valuable clinical decisions regarding high-risk revascularization procedures.

## References

- [1] Huang PJ, Lin LC, Yen RF, et al. Accuracy of biphasic response, sustained improvement and worsening during dobutamine echocardiography in predicting recovery of myocardial dysfunction after revascularization: comparison with simultaneous thallium-201 reinjection SPECT. *Ultrasound Med Biol* 2001; 27:925-931.
- [2] Allman KC, Shaw LJ, Hachamovitch R, et al. Myocardial viability testing and impact of revascularization on prognosis in patients with coronary artery disease and left ventricular dysfunction: a meta-analysis. *J Am Coll Cardiol* 2002; 39:151-158.
- [3] Underwood SR, Bax JJ, vom Dahl J, et al. Imaging techniques for the assessment of myocardial hibernation: report of a study group of the European Society of Cardiology. *Eur Heart J* 2004; 25:815-836.
- [4] Bax JJ, Poldermans D, Schinkel AF, et al. Perfusion and contractile reserve in chronic dysfunctional myocardium: relation to functional outcome after surgical revascularization. *Circulation* 2002; 106:114-118.
- [5] Baer FM, Voth E, Schenider CA, Theissen P, Schicha H, Sechtem U. Dobutamine gradient echo MR: a functional and morphologic approach to the detection of residual myocardial viability. *Circulation*. 1995;91:1006-1015.
- [6] Shan K, Constatine G, Sivananthan M, Flamm S. Role of cardiac magnetic resonance imaging in the assessment of myocardial viability. *Circulation* 2004; 109:1328-1334.
- [7] Mahrholdt H, Wagner A, Holly TA, et al. Reproducibility of chronic infarct size measurement by contrast-enhanced magnetic resonance imaging. *Circulation* 2002; 39:1151-1158.
- [8] Wagner A, Mahrholdt H, Regenfus M, et al. Contrast-enhanced MR and routine single photon emission computed tomography (SPECT) perfusion imaging for detection of subendocardial myocardial infarcts: an imaging study. *Lancet* 2003; 361:364-379.

- [9] Kim RJ, Wu E, Rafael A, et al. The use of contrast-enhanced magnetic resonance imaging to identify reversible myocardial dysfunction. *N Engl J Med* 2000; 343:1445-1453.
- [10] Cerqueira MD, Weissman NJ, Dilsizian V, et al. Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart. *Circulation* 2002; 105:539-542.
- [11] Mahrholdt H, Wagner A, Holly TA, et al. Reproducibility of chronic infarct size measurement by contrast-enhanced magnetic resonance imaging. *Circulation* 2002; 106:2322-2327.
- [12] Knuesel PR, Nanz D, Wyss C, et al. Characterization of dysfunctional myocardium by positron emission tomography and magnetic resonance: relation to functional outcome after revascularization. *Circulation* 2003; 108:1095-1100.
- [13] Tseng WY, Liao TY, Wang JL. Normal systolic and diastolic functions of the left ventricle and left atrium by cine magnetic resonance imaging. *J Cardiovasc Magn Reson* 2002; 4:443-457.
- [14] Nelson C, McCrohon J, Khafagi F, et al. Impact of scar thickness on the assessment of viability using dobutamine echocardiography and thallium single photon emission computed tomography: a comparison with contrast-enhancement magnetic resonance imaging. *J Am Coll Cardiol* 2004; 43: 1248-1256.
- [15] Klein C, Nekolla SG, Bengel FM, et al. Assessment of myocardial viability with contrast-enhanced magnetic resonance imaging: comparison with positron emission tomography. *Circulation* 2002; 105:162-167.
- [16] Zamorano J, Delgado J, Almeria C, et al. Reason for discrepancies in identifying myocardial viability by thallium-201 redistribution, magnetic resonance imaging and dobutamine echocardiography. *Am J Cardiol* 2002; 90:455-459.
- [17] Rogers WJ, Kramer CM, Geskin G, Hu Y-L, Theobald TM, Vido DA, Petruolo S, Reichek N. Early contrast-enhanced MRI predicts late functional recovery after reperfused myocardial infarction. *Circulation*. 1999;99:744-750.
- [18] Wu KC, Zerhouni EA, Judd RM, Lugo-Olivieri CH, Barouch LA, Schulman SP, Blumenthal RS, Lima JAC. Prognostic significance of microvascular obstruction by magnetic resonance imaging in patients with acute myocardial infarction. *Circulation*. 1998;97:765-772.
- [19] Baer FM, Theissen P, Schneider CA, Voth E, Sechtem U, Schicha H, Erdmann E. Dobutamine magnetic resonance imaging predicts contractile recovery of chronically dysfunctional myocardium after successful revascularization. *J Am Coll Cardiol*. 1998;31:1040-1048.
- [20] Wendland MF, Saeed M, Arheden H, Gao D-W, Canet E, Bremerich MD, Dae MW, Higgins CB. Toward necrotic cell fraction measurement by contrast-enhanced MRI of reperfused ischemically injured myocardium. *Acad Radiol*. 1998;5(suppl):S45-S46.
- [21] Sharma R, Sharma A. Advances in Magnetic Resonance Angiography, Chapter 3, 2004: pp 119-187 in: A Hand Book in Medical Imaging, Editors: Suri, Wilson and Laxminarayana, Springer Science, USA.
- [22] Sakuma H, Blake LM, Amidon TM, OiSullivan M, Szolar DH, Furber AP, Bernstein MA, Foo MKF, Higgins CB. Coronary flow reserve: noninvasive measurement in humans with breath-hold velocity-encoded cine MR imaging. *Radiology*. 1996;198:745-750.
- [23] Hundley WG, Lange RA, Clarke G, Meshack BM, Payne J, Landau C, McColl R, Sayad DE, Willet DWL, Willard JE, Hillis LD, Peshock RM. Assessment of coronary arterial flow and flow reserve in humans with magnetic resonance imaging. *Circulation*. 1996;93:1502-1508.
- [24] Selvanayagam JB, Petersen SE, Francis JM, et al. Effects of off-pump versus on-pump coronary surgery on reversible and irreversible myocardial injury. A randomized trial using cardiovascular magnetic resonance imaging and biochemical markers. *Circulation* 2004; 109:345-350.
- [25] Wu YW, Huang PJ, Su MY, Lin LC, Yen RF, Chen YS, Lee CM, Yu HY, Ting Y, Tzen KY, Tseng WYI. Myocardium Viability Assessed by Late Enhancement Magnetic Resonance in Patients with Severe Ischemic Heart Failure: A Comparison with Thallium SPECT and Dobutamine Echocardiography. *World Heart J*. 2005; ---- --.
- [26] Duerinckx AJ. Myocardial Viability Using MR Imaging: Is It Ready for Clinical Use? *Am. J. Roentgenol.*, June 1, 2000; 174(6): 1741 - 1743.
- [27] Higgins CB. Prediction of myocardial viability by MRI *Circulation*. 1999;99:727-729.





## EDITORIAL

# Gene Therapy for Heart Disease

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Insights into the molecular basis of cardiac disease have led to the development of gene therapy as a possible treatment mode. The genetic understanding of some disorders such as vascular disease, including atherosclerosis, hypertrophic cardiomyopathy, prolonged Q-T interval, and heart failure has rendered them to gene manipulation to improve both structure and function. Direct gene transfer for hepatic regulation of lipoprotein metabolism holds promise for human gene therapy in familial hypercholesterolaemia.

Cardiovascular disorders are not single gene diseases. Multiple genes appear to be associated in cardiovascular disease, but not all studies of associated genes can be replicated. [1] As compared with hereditary single-gene disorders, cancers, or AIDS, the risk-to-benefit for cardiovascular patients is higher. Identification of candidate genes for treatment has been difficult. However, potential candidate genes have, and are still being, identified. There have been improvements in catheter design and delivery, gene expression, and vector pharmacokinetics, so that gene therapy now appears to be on the way to becoming a therapeutic option for cardiovascular patients. [2]

Whereas clinical cardiology earlier focused on angiographically demonstrable stenotic lesions for addressing myocardial infarction and unstable angina, basic research is now highlighting the role of inflammatory mediators in these disease states. Some of the cytokines may be amenable to genetic manipulation. Genetic understanding of atherosclerosis can help modify this process also. Further, myocardial damage following infarction is free-radical mediated. Genes and proteins involved have been identified giving hope of a favourable outcome by their manipulation.

Coronary restenosis following balloon angioplasty is well known. The insertion of drug-eluting stents has improved outcomes, but restenosis still occurs. Gene therapy may be a useful approach here, [3] as also for cardiac pacemaking disorders, despite the advancements in implantable pacemakers/defibrillators. [4]

The identification of candidate genes is only the first step. Vectors are needed to deliver the genetic material to the target cells. The ideal vector should be synthetic, non-immunogenic, and capable of targeting the desired cell. Plasmid liposomes have limited efficiency for gene transfer. Retroviruses not only have limited gene transfer efficiency, but also have the associated risk of transformation. Adenoviruses are highly efficient at gene transfer and can transduce quiescent cells. However, even second-generation adenoviruses provoke a local inflammatory response, and the transgene expression is only transient. In humans who have been preimmunised with native adenoviruses, this method of delivering gene therapy may be ineffective. Further, the safety of gene therapy using adenoviral vectors has been questioned, particularly in diseases such as coronary restenosis, which are, by and large, rarely lethal. [5]

Recently interventional magnetic resonance imaging (MRI) has been used for providing real time guidance for gene and cell delivery into the heart, and for assessing heart modeling. Genes, proteins, and cells for tissue engineering can be accurately delivered by intramyocardial injection. [6] This may be a significant advance in the delivery of genes into

the cardiac myocytes. The vascular smooth muscle and endothelial cells are more easily accessible through the percutaneous intra-arterial route, and with improvements in design of delivery catheters targeting these cells may be facilitated further.

The problem that remains after this is how long will the gene expression last in the relevant cells? As of now, the duration does not exceed a few weeks, which leads to the question—what next? Also, many of the studies have been done mainly on animals. Would the results in humans be the same?

It appears that, whereas we may not have actually “arrived” there, we are probably well on our way to developing gene therapy into a useful therapeutic option in the practice of cardiovascular medicine.

## References

- [1] Sturm A.C.: Cardiovascular genetics: are we there yet? *Journal of Medical Genetics* 2004; 41:321-323.
- [2] Nabel E.G.: Gene therapy for cardiovascular disease. *Circulation* 1995; 91:541-548.
- [3] O’Sullivan M.J.; Bennet M.R.: Gene therapy for coronary restenosis: is the enthusiasm justified? *Heart* 2001; 86: 491-493.
- [4] Edelberg J.M.; Huang D.T.; Josephson M. E. et al: Molecular enhancement of porcine cardiac chronotropy. *Heart* 2003; 86: 559-562.
- [5] O’Sullivan M.J.; Bennet M.R.: Gene therapy for coronary restenosis: is the enthusiasm justified? *Heart* 2001; 86: 491-493.
- [6] Barash I.M.; Leor J.; Feinberg M.S.: Interventional magnetic resonance imaging for guiding gene and cell transfer in the heart. *Heart* 2004; 90: 87-91.



## EDITORIAL

# Dietary/Blood Cholesterol and Coronary Heart Disease

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In some individuals, dietary cholesterol influences blood cholesterol when tested clinically and, therefore, it has long been perceived as a causal factor in the development of atherosclerosis and CHD. The time has come when this long-held perception is scientifically examined in the light of emerging knowledge.

Dietary and blood cholesterol are identical interchangeable molecules and dietary cholesterol is not essential for homeostasis and health in young healthy men and women. In omnivorous men and women, it is estimated that, on average,  $\pm 2/3^{\text{rd}}$  of plasma cholesterol is produced by the liver and  $\pm 1/3^{\text{rd}}$  is derived from the diet and biliary salts. There also seems to be a daily intake threshold ( $\pm 500$  mg) above which dietary cholesterol has no more clinically observable influence on blood cholesterol.

The clinically established dietary-blood cholesterol relationship does not survive the test of time. Epidemiological surveys conclude to a null relationship between dietary and blood cholesterol and, therefore, to a null relationship between dietary cholesterol and coronary heart disease (CHD) (Dawber et al., 1982). This epidemiological observation comes in strong support to the otherwise undisputed scientifically established evidence that dietary cholesterol efficiently suppresses – just as efficiently as blood cholesterol – the body's own synthesis.

The way the overall dietary pattern affects blood cholesterol is phenotype-driven. It depends on genetic, environmental/constitutional, and developmental factors, which basically influence cholesterol's absorption, circulation/distribution, and excretion. Re-analyzing clinical and epidemiological studies carried out in the last quarter of the last century for confounding factors, McNamara (2002) has provided evidence that dietary saturated fatty acids and blood cholesterol could well be leading factors in CHD; however, Okuyama (2000) has raised the interesting point which saturated fatty acid intake and high blood cholesterol in western industrialized countries are associated with high intake of vegetable and animal fats abnormally rich in

omega-6 fatty acids. At the same time, the diet of Inuits, known to have high cholesterol-saturated fats index (CSI), but also to contain high amount of omega-3 fatty acids, has been reported to protect against CHD.

It, therefore, seems reasonable to suggest that the blood cholesterol-dietary saturated fats index is a marker for CHD in western industrialized civilizations for the main reason that their dietary lipid patterns are characterized by artificially high omega-6:omega-3 fatty acids ratios.

## **Epidemiological Evidence**

As a result of the last 40-years of generally extended phobia around the classical dietary cholesterol-saturated fats index (CSI), animal fat has become synonymous with CHD. This is at least the present perception that the consumer and, to a large extent, health authorities, medical doctors, dieticians and opinion leaders have of the dietary lipids/heart diseases relationship. Classic recommendations for the prevention of these diseases, apparently established in the sixties, were that the intake of cholesterol should be reduced and the polyunsaturated:saturated (P:S) ratio of dietary fatty acids should be increased. Basically, a decrease intake of animal fats was advised, whereas increased intake of hydrogenated vegetable margarine and high-linoleic acid vegetable oils was recommended.

Despite a failure to demonstrate the effectiveness of this recommendation, the recommendation has continued until very recently in all industrialized countries, in the hope that a long-term follow-up trial would prove the effectiveness of such recommendations (Okuyama, 2000). However, long-term follow-up studies performed in the West and in Japan conclusively demonstrate the following two facts:

- Those with higher blood cholesterol survive longer, possibly due to decreased cancer mortality, decreased mortality from infectious diseases and/or decreased apoplexy
- Blood cholesterol is not an independent risk factor for atherosclerosis and CHD.

## **Dietary Cholesterol – A Beneficial Nutrient?**

The ingestion of dietary cholesterol translates into a parallel increased synthesis of apo E in the body's tissues, starting with the intestinal enterocytes. The increased apo E level concomitantly increases cholesterol secondary circulation (reverse cholesterol transport) throughout the body through participation of HDL-1 and HDLc while, at the same time, it reduces the body's own cholesterol synthesis in the liver.

Cholesterol is an important component of cell membranes. It is well known that macrophages synthesize and excrete large amounts of apo E at sites of injury as chemo-attractants for tissue- and cell-repairing cholesterol.

On the other hand, this sterol is so crucial to life and cell-functioning that man's brain has developed an almost independent biological manufacturing process for the cholesterol it requires in a typical omega-3 rich environment.

The body's own synthesis of cholesterol is hormone-sensitive and energy-demanding. To some extent, it shares common features with other animal-derived nutrients such as long chain polyunsaturated fatty acids or LC-PUFAs. Therefore, there are scientific reasons to support the hypothesis that dietary cholesterol might be needed for normal metabolism and more so infants and aging adults.

We can therefore conclude that dietary cholesterol has all the potential of being beneficial to human health – protecting it against infection, cancer, and degenerative diseases – and that it is the individual phenotype that defines how dietary/blood cholesterol circulates and is distributed in and excreted out of the body. The classical clinical blood cholesterol profile (TC, LDL, HDL) is just an in-print of this individual specific phenotype-dietary/blood cholesterol interaction at a certain time of our life. In no such case, can this temporary signature be assimilated to a risk factor for CHD and degenerative diseases per se.

There is epidemiological evidence to support the view that dietary cholesterol might be beneficial to nascent life and longevity, and that it is not related, per se, to health diseases in normal adults:

- Mother's milk provides 18-mg cholesterol per 100-ml milk or 27-mg cholesterol per kg body weight (150-ml) to the breast-fed infant, in association with essential and conditionally essential fatty acids
- Among those highest in age followed for 10 years in the Netherlands (Weverling-Rijnsburger et al., 1997), no significant correlation was found between serum cholesterol level and CHD mortality. Instead, both cancer mortality and mortality from infectious diseases were higher when serum CHL was lower, and all causes of mortality were negatively correlated with serum cholesterol level. A similar conclusion was obtained when people older than 70 years were followed for 10 years in the USA (Krumholz et al., 1994)
- The 24-industrialized countries' epidemiological data suggest that countries where egg consumption is the highest are those where the risk of fatal cardiovascular diseases is the lowest (McNamara, 2000). Interestingly, the four countries (France, Spain, Japan, Mexico) with the highest egg consumption and the lowest rate of CVD-mortality are also those where the average dietary lipid profile is the most perfectly balanced (ideal dietary  $\omega_6:\omega_3$  ratio from greens and game) and where the average consumption of antioxidant-rich foods (wine, fruits, vegetables, spices,...) is the highest. The Japanese consume more than 340 eggs per capital per year, twice the amount reported for the United Kingdom, but the incidence of

heart disease in Japan is one of the lowest in the world (Simopoulos, 1991).

These observations run counter to the long held perception that dietary/blood cholesterol is a causal factor in the genesis of CHD.

Based on "Common Statement" emerging from the International Congress on the Columbus® Concept; edited by Artemis P. Simopoulos.

## References

- Dawber TR, Nickerson RJ, Brand FN, Pool J. Eggs, serum cholesterol, and coronary heart disease.*Am J Clin Nutr* 1982,36:617-25.
- Krumholz HM, Seeman TE, Merrill SS, et al. Lack of association between cholesterol and coronary heart disease mortality and morbidity and all-cause mortality in persons older than 70 years. *JAMA* 1994,272:1335-1340.
- McNamara DJ. Dietary cholesterol and atherosclerosis. *Biochim Acta* 2000,1529:310.
- McNamara DJ. Egg, plasma cholesterol and heart disease risk.Eggs and Health Promotion.Watson RR(ed), Iowa State Press 2002,8,p71-81.
- Okuyama H, Fuyii Y, Ikemoto A.n-6/n-3 ratio of dietary fatty acids rather than hypercholesterolemia as the major risk factor for atherosclerosis and coronary heart disease. *J Health Sci* 2000,46(3):157-177.
- Simopoulos AP. Omega-3 fatty acids in health and disease and in growth and development. *Am J Clin Nutr* 1991, 54:438-463.
- Weverling-Rijnsburger AW, Blauw GJ, Lagaay AM, Knook DL, Meinders AE, Westendorp RG.Total cholesterol and risk of mortality in the oldest old. *Lancet* 1997,350:1119-1123.





## ARTICLE

# Revascularization in Acute Myocardial Infarction Using Percusurge in Distal Protection Acronym: RAPID

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## Abstract

### Background

The PercuSurge Guardwire Plus Temporary Occlusion And Aspiration System is underused due to the misconceptions that it is less user-friendly and makes PCI in AMI complicated and time consuming. This prospective study evaluates the efficacy of the PercuSurge device in primary/rescue PCI and focuses on the actual procedural time involved with or without its use.

### Methods

It is a single center prospective study in 67 AMI patients undergoing PCI for thrombotic lesions. The patients were divided randomly into two groups, one where PercuSurge was used during PCI (n=30) and the other where it was not (n=37). In addition to the conventional demographic and angiographic parameters including TIMI flow and TMP grade, the time involved in various steps of the PCI were recorded. We considered the total procedural time as the time involved after the engagement of the guide catheter to the attainment of the desired end point.

### Results

PercuSurge use showed a significantly greater achievement of TIMI III flow and TMP III grade ( $p<0.01$ ). The total procedural time for PCI was significantly reduced with PercuSurge use ( $25.01 \pm 2.17$  min vs.  $31.98 \pm 2.52$  min without its use,  $p<0.05$ ). The time required after wire crossing to reach stent placement and to achieve TIMI III flow was also significantly less with PercuSurge use. Further, use of PercuSurge significantly reduced the time after PTCA balloon inflation / stent placement till optimal TIMI flow achievement ( $17.37 \pm 2.21$  min vs.  $24.41 \pm 2.5$  min without its use,  $p<0.05$ ). The incidences of no/slow reflow were significantly reduced ( $p<0.001$ ) with use of PercuSurge, thus requiring less administration of intracoronary vasodilators and GP IIb/IIIa receptor inhibitors.

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## Conclusions

Recent results of EMERALD trial have shown that PercuSurge increases the procedural time. However, our experience seems to have shown significant time and TMP advantage (as seen in the RUBY registry). More studies need to be done in focused and experienced centres.

**Key words:** PercuSurge, Distal protection, acute myocardial infarction, procedural time

## Abbreviations

MACE:Major Adverse Cardiac Events

SVG: Saphenous Vein Graft

NTG: Nitroglycerin OR Glyceryl Trinitrate

ACC: American College of Cardiology

Re MI: Recurrent Myocardial Infarction

## Introduction

The efficacy of primary angioplasty in acute myocardial infarction (AMI) has been well proven by randomized clinical trials [1]. Increasing evidence has suggested that microvascular debris and/or platelet-thrombus plays a crucial role in embolization to the microvasculature [2, 3], which subsequently leads to the no reflow phenomenon [2, 4] during elective or primary percutaneous coronary interventions (PCI). This distal embolization of plaque or thrombotic debris during angioplasty in AMI results in infarct extension and high periprocedural mortality [5]. Recently, some studies demonstrated that infarct related arteries (IRAs) usually contain high burden thrombus formation (HBTF) [6, 7] and lipid pool like contents [8, 9]. Several distal protection devices have been developed to prevent distal embolization during angioplasty, [10-14] and their effectiveness has been reported [15-17]. The PercuSurge Guardwire Plus Temporary Occlusion and Aspiration System (Medtronic AVE, Santa Rosa, CA) is one such device designed to provide protection of the distal microcirculation during percutaneous intervention by temporarily occluding the distal vessel, thereby facilitating aspiration of dislodged atheromatous and thrombotic material before it can reach the arteriolar and capillary beds. Some studies have reported favorable results with this device as an adjunct to percutaneous intervention in saphenous vein graft,

[18-20] carotid artery, [21-23] and the coronaries. Trials like SAFE and SAFER have already proven the efficacy of this device with reduction in MACE, MI and mortality in patients with SVG lesions [19, 20].

Large-scale trials like EMRALD and RUBY registry have investigated the efficacy of the device in patients with AMI. Interim conclusions from RUBY registry have shown that the device has a high delivery success rate and a large number of patients were found to achieve TIMI III after the procedure. However, the results from EMERALD trial, which is the largest one in AMI, failed to show a beneficial effect from the use of distal embolic protection devices. On the contrary, it showed that the Guardwire device was not associated with an improvement in the primary endpoints of post-procedure ST resolution or final infarct size. Further, it concluded that the procedural duration was significantly longer in the Guardwire arm of the study than without its use. Thus, there is a possibility that using these devices makes the intervention more complicated and time consuming as compared with conventional PCI. In this study we investigated the efficacy of this device in the setting of primary / rescue PCI in AMI. Also, the time required in the procedure with and without the use of PercuSurge was compared.

## Materials and Methods

### Study Population

67 consecutive AMI patients with angiographically detected thrombotic lesions who were to undergo primary/rescue PCI within 24hrs of the onset of chest pain were enrolled for the study from September 2003 to December 2003. The patients were randomly divided into two groups depending on whether PercuSurge was used or not during PCI. In all, Guardwire was used in 30 patients. The remaining 37 patients formed the control group.

### Study Protocol

It was a single centered, random, open clinical trial. It was non-blind and based on parallel group

design. Informed consent was obtained from all the patients. After recording the baseline demographic and hemodynamic parameters, the angiographic parameters were also assessed. PCI was performed according to the conventional methods. An angiographic criteria of <30% residual stenosis was used to determine the end point of PCI. The incidences of no/slow reflow as well as the use of intracoronary vasodilators like adenosine, sodium nitroprusside, NTG, and GP IIb/IIIa receptor antagonists were also recorded. TIMI flow and TMP grade after PCI were assessed and the total procedural time and the time for individual steps of the PCI were recorded.

### Device Description

The description of the Guardwire system has been well elaborated in a recent article.[24] It consists of four principal components: the Guardwire Plus Temporary Occlusion Catheter, the Micro Seal Adaptor, the Export Catheter, and the EZ flator inflation device (Figure 1). The Guardwire catheter is a novel 0.014 inches balloon-on-a-hypotube-wire catheter with a distal elastomeric occlusion balloon.

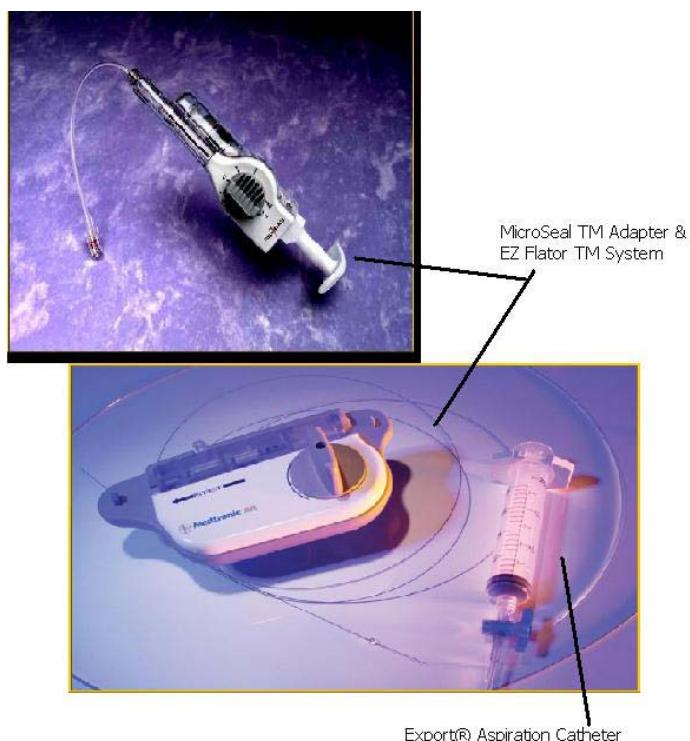


Figure 1: Components of PercuSurge device

### Procedure Description

The femoral arterial approach was used in all cases. After a proper guiding catheter was engaged to the target vessel, the Guardwire (or conventional soft guidewire in cases where the device was not used) was first attempted to cross the culprit lesion. If there was difficulty passing the Guardwire, a conventional guidewire was passed over which the Guardwire was crossed using the buddy wire technique. The

Guardwire was advanced till the distal balloon was positioned a few centimeters beyond the target lesion. To protect the side branches it was important not to push the Guardwire too far distal from the lesion. The Guardwire catheter then served as an adequate guidewire for delivery of dilatation balloons and a multitude of stents. After inflating the Guardwire distal balloon, PCI was performed according to the conventional methods. The distal balloon was kept inflated during the treatment of the culprit lesion.

Before deflating this balloon, the liberated debris (after PTCA balloon inflation and stent implantation) was aspirated through the Export aspiration catheter.

### *Angiographic Analysis*

2 independent cardiologists unaware of the patients' medical histories and details reviewed coronary angiograms separately, to avoid inter observer bias. The degree of perfusion was evaluated according to Thrombolysis in Myocardial Ischemia (TIMI) criteria. Good collateral flow was defined as grade 2 or 3 (discussed below). Angiographic thrombus was defined as a filling defect seen in multiple projections surrounded by contrast and in the absence of calcification. Angiographic distal embolization was defined as the angiographic cut-off of a distal branch or vessel at any point during the procedure.

### *Procedural Time*

Time in minutes required for various steps of the PCI procedure was recorded. The time count was started after the guiding catheter was engaged and viewed under fluoroscopy to ensure that it was in place. The rest of the time frames were calculated considering the guiding catheter time as zero or start time of the procedure. Various time periods like time for crossing of the lesion by the wire; time required to achieve PTCA balloon inflations; time to reach stent implantation, and time to achieve optimal TIMI flow were recorded separately in addition to the total procedural time. The *total procedural time*, in our trial, consists precisely of the time period from the first cinefilm of guide catheter engagement to the last one when the final TIMI flow is achieved.

### *Study Endpoints*

The primary endpoint of this study was to evaluate the presence of distal embolization, which was demonstrated by Thrombolysis in Myocardial Infarction (TIMI) flow and Myocardial Blush grade. A secondary endpoint was to evaluate the clinical

performance of the Guard-wire system combined with Plain-old balloon angioplasty (POBA) and/or stenting in AMI patients during emergency PCI. This included immediate technical device success, angiographic success and procedural success.

### *Assessment of Blood Flow*

#### ***Thrombolysis in Myocardial Infarction (TIMI) Flow Grades***

The culprit lesion was determined by its anatomical location and its perfusion characteristics according to Thrombolysis in Myocardial Infarction (TIMI) classification for flow through the infarct related vessel:[25]

**TIMI flow 0** was assigned if there was no anterograde flow beyond the point of occlusion or there was no visible filling of any collateral channel.

**TIMI I** flow was designated if there was penetration, but without perfusion, or only minor perfusion, i.e., contrast material passed beyond the area of obstruction, but "hung up" and failed to opacify the entire coronary bed distal to the obstruction for the duration of the cineangiographic filming sequence. There was filling by means of collateral channels of side branches of the vessel but without any dye reaching the epicardial segment of that vessel.

Blood flow was graded as **TIMI II** when there was partial or mild perfusion. The passing of the obstruction by the contrast material or its rate of clearance from the distal bed (or both) was perceptibly slower than its entry into or clearance from comparable areas not perfused by previously occluded vessels. Partial filling occurred via collateral channels of the epicardial segment of the vessel.

**TIMI III** flow was assigned for complete and good perfusion as visualized by complete filling of the vessel. Anterograde flow into the bed distal to the obstruction occurred as promptly as anterograde flow into the bed proximal to the obstruction, and clearance of contrast material from the involved bed was as rapid as clearance from an uninvolved bed in the same vessel or an opposite artery.

### **Myocardial Blush Grade/ TIMI Myocardial Perfusion (TMP)**

Perfusion can be defined as tissue blood flow at the capillary level. Perfusion of the myocardium can be categorized using the TIMI myocardial perfusion (TMP) classification described below: [26]

Myocardial blush was graded as **0** when there was failure of the dye to enter the microvasculature. There was either minimal or no ground-glass appearance (“blush”) or opacification of the myocardium in the distribution of the culprit artery, indicating lack of tissue-level perfusion.

TMP grade **1** was assigned when the dye slowly entered but failed to exit the microvasculature. There was ground-glass appearance (“blush”) or opacification of the myocardium in the distribution of the culprit lesion that failed to clear from the microvasculature, and dye staining was present on the next injection.

TMP grade was considered as **2** when there was delayed entry and exit of dye from the microvasculature. There was ground-glass appearance (“blush”) or opacification of the myocardium in the distribution of the culprit lesion that was strongly persistent at the end of the washout phase (i.e., dye was strongly persistent after 3 cardiac cycles of the washout phase and either did not, or only minimally, diminished in intensity during washout).

TMP grade **3** was designated when there was normal entry and exit of dye from the microvasculature. There was ground-glass appearance (“blush”) or opacification of the myocardium in the distribution of the culprit lesion that cleared normally. It was either gone or only mildly/moderately persistent at the end of the washout phase (i.e., the dye was gone or mildly/moderately persistent after 3 cardiac cycles of the washout phase and diminished noticeably in intensity during this phase), similar to that in an uninvolved artery. Blush that was only of mild intensity throughout the washout phase but faded minimally was also classified as grade 3.

### **Device Success**

Device success was defined as successful deployment of the Guardwire catheter, occlusion of the distal flow and performance of aspiration.

### **Angiographic Success**

Angiographic success was defined as residual lumen diameter (stenosis) < 30% and TIMI flow grade 3 after the intervention.

### **Procedural Success**

Procedural success was defined as angiographic success without the occurrence of periprocedural adverse events.

### **Periprocedural Adverse Events**

Periprocedural adverse events were defined as distal embolization; spasm; no/slow reflow during the procedure, and all causes of death, including new Q wave or non Q wave AMI, emergent CABG, or repeat percutaneous target vessel revascularization within 3 days of the index procedure.

### **No Reflow**

Coronary occlusion leads to cellular necrosis and myocardial damage. During a short period of occlusion, a variable number of myocytes may become necrotic while the microvascular network is still intact. If coronary occlusion is prolonged, the microvasculature shows loss of its anatomic integrity. [27]. At the time of coronary reopening, myocardial reperfusion is achieved only in areas with anatomically preserved microvasculature, where as reflow does not occur in myocardium with extensive microvascular damage. The no reflow phenomenon is therefore associated with relatively more extensive necrosis and, as a consequence, is a predictor of poor regional and global contractile function. Angiographic ‘no reflow’ was defined as the cessation of flow into the distal coronary circulation of the treated vessel with a to and fro contrast movement after intervention (TIMI grade 0 or 1 flow in patients with prior TIMI grade 2 or 3, or TMP grade 0 or 1) not attributable to high grade stenosis or spasm of the original target vessel.

### **Statistical Analysis**

Values have been expressed as mean  $\pm$  SEM (standard error of mean). Continuous data were compared using unpaired Student’s t test. Independent variables included were age, sex, classic coronary risk

factors, angiographic lesion characteristics and interventional strategies and were compared by non-parametric test. A p value <0.05 was considered to be statistically significant.

## Results

### *Baseline Demographic, Hemodynamic and Biochemical Characteristics*

Out of the 67 patients undergoing primary/rescue PCI, Guardwire system was used in 30 patients.

Baseline demographic, hemodynamic characteristics, and risk factor presentation (Table 1) were nearly identical in both the arms indicating symmetry in the study design. However, group II had more diabetic patients (41%) as compared to group I (20%). The Guardwire system was successfully deployed, distal balloon occlusion established and aspiration completed in all the cases in which PercuSurge was used (100% device success) without any complications. Group I consisted of 30 patients in which PercuSurge was used and Group II consisted of 37 patients in which PercuSurge was not used.

**Table 1. Baseline Demographic and Hemodynamic Characteristics of patients**

	Group I (n=30)	Group II (n=37)
<i>Demographic Characteristics</i>		
Age	55.17 ± 2.19	56.16 ± 1.86
% Males	90%	95%
% Females	10%	5%
<i>Risk Factors</i>		
Smoking	17%	19%
Tobacco Consumption	7%	27%
Diabetes	20%	41%
Hypertension	20%	28%
Hyperlipidemia	3%	8%
Significant Family History	23%	27%
<i>Vital Signs</i>		
Systolic BP (mm Hg)	117 ± 4	118 ± 3
Diastolic BP (mm Hg)	75 ± 3	72 ± 2
Heart Rate (beats/min)	84 ± 4	87 ± 3
<i>Haematological &amp; Biochemical investigations</i>		
Hb (Gm %)	13.05 ± 0.43	13.37 ± 0.26
TC (counts x 10 <sup>9</sup> /L)	11.740 ± 0.736	11.281 ± 0.678
PC (counts x 10 <sup>9</sup> /L)	257.522 ± 15.938	267.939 ± 11.949
Random blood sugar (mmol/L)	6.69 ± 0.89	10.89 ± 0.96
Urea (mg/dl)	34.85 ± 4.64	29.83 ± 2.13
Creatinine (μmol/L)	106.08 ± 9.72	96.35 ± 3.53
Na <sup>+</sup> (mmol/L)	138.2 ± 0.99	137.63 ± 0.74
K <sup>+</sup> (mmol /L)	4.23 ± 0.13	4.13 ± 0.09

Data expressed as Mean ± SEM; % patients

Group I: PCI with use of PercuSurge device

Group II: PCI without use of PercuSurge device

### *Angiographic and Lesion Characteristics and PCI Details*

Most of the patients in both the groups had single vessel disease (80% in group I and 68% in group II). Left anterior Descending artery (LAD) was found to be

the culprit coronary in maximum number of cases (53% in group I and 62% in group II). 23% of patients in group I and 27% in group II had a type B1 lesion whereas 70% in group I and 54% in group II depicted type B2 lesion. Type C lesion was present in 7% of group I and 19% of group II patients. Thrombus was

present in all the cases and the culprit artery was totally occluded in 73% and 65% patients in groups I and II respectively. Predilatation was done before stenting of the artery in 47% and 57% of cases in groups I and II respectively. However, plain old balloon angioplasty

(POBA) as well as direct stenting were also performed in rest of the cases at the cardiologist's discretion (Table 2). Angiographic and procedural success was achieved in 100% of patients in whom Guardwire was used.

**Table 2. Lesion Characteristics and Procedure Details**

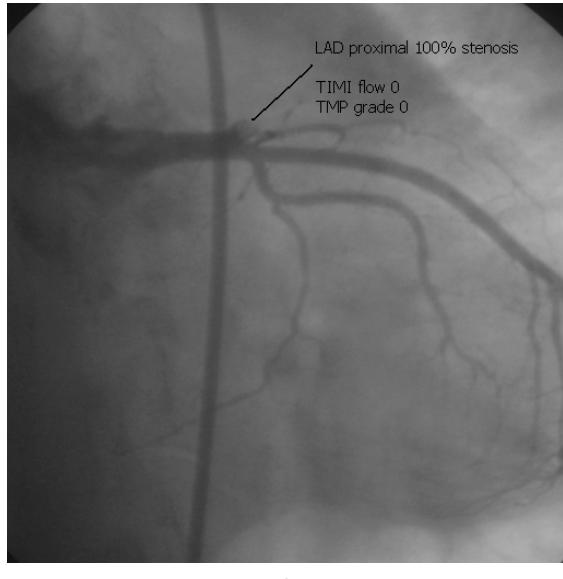
Parameters		Group I (n=30)	Group II (n=37)
<i>Angiographic Characteristics</i>			
LVEF (%)		33.44 ± 4.69	41.22 ± 4.79
Single Vessel Disease (SVD)		80%	68%
Double Vessel Disease (DVD)		13%	19%
Triple Vessel Disease (TVD)		7%	13%
Culprit Coronaries	LAD	53%	62%
	RCA	43%	24%
	LCX	4%	14%
Area of the artery having the lesion	Proximal	70%	62%
	Mid	10%	3%
	Distal	10%	5%
	Prox. / Mid	7%	8%
	Ostial	4%	-
<i>Lesion Details</i>			
Lesion Type	A	-	-
	B1	23%	27%
	B2	70%	54%
	C	7%	19%
Lesion Characteristics	Thrombus	100%	100%
	Total Occlusion	73%	65%
	Calcification	-	8%
	Ulcerated	3%	3%
<i>Procedure Details</i>			
Plain Balloon PTCA		20%	35%
Predilatation		47%	57%
Direct Stenting		33%	8%
Type of Stents Used	Cypher	17%	19%
	Driver	26%	8%
	S7	13%	19%
	S670	10%	8%
	BE2	10%	5%
	Others	-	5%
Incidences of No Reflow (p<0.001)		30% (9)	84% (31)
% patients in whom TIMI III achieved (p=0.003)		87% (26)	49% (18)
% patients in whom TMP III achieved (p<0.001)		80% (24)	14% (5)
% patients in whom GP IIb/IIIa inhibitors used (p=0.048)		43% (13)	70% (26)
% patients with use of IC drugs	Adenosine	53 % (16)	86% (32) (p=0.007)
	SNP	53% (16)	76% (28) (p=0.098)
	NTG	20% (6)	40% (15) (p=0.124)

Data expressed as Mean ± SEM; % patients

Group I: PCI with use of PercuSurge device; Group II: PCI without use of PercuSurge device

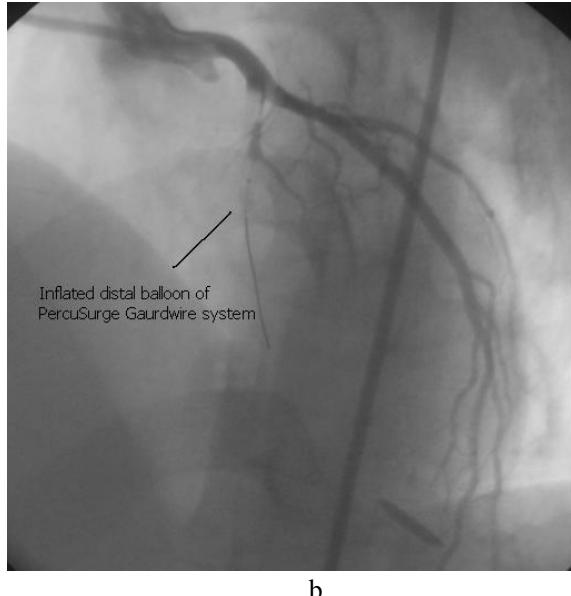
### *Periprocedural Adverse Event, Intracoronary Agents and Myocardial Flow Assessment*

Incidence of no/slow reflow occurred in 30% of cases in group I and in 84% in group II after predilatation / direct stenting or POBA (Table 2). The higher incidences of no reflow may be due to the higher thrombotic burden in group II. This debris was aspirated in group I by the PercuSurge device, leading to fewer incidences of no/slow reflow and other periprocedural events in this group. 87% of patients achieved TIMI III and 80% TMP III in group I whereas only 49% achieved TIMI III and 14% achieved TMP III in group II without the use of PercuSurge (Table 2). Figure 2 (a, b, c, d) and Figure 3(a and b) show the angiographic views of PCI done with and without the use of PercuSurge and depict the improvement in TIMI flow and TMP grade achieved with the use of Guardwire system as compared to PCI without its use. Figure 2(a) and Figure 3(a) shows the LAD artery with stenosis before PCI was done. In figure 2(b), the distal balloon of PercuSurge device is shown inflated. Figure 2(c and d) show the artery after PCI was done. TIMI flow III and TMP grade 3 were obtained with use of PercuSurge as seen in these figures. Figure 3(b) shows that adequate TIMI and TMP flow were not obtained after PCI wherein PercuSurge was not used. In order to optimize the TIMI flow and the myocardial perfusion when no reflow occurred, intracoronary (IC) boluses of vasodilators that included adenosine, sodium nitroprusside as well as NTG were administered. Further, a concomitant use of GP IIb/IIIa antagonists was required in significantly less patients if PercuSurge was used, based on the ACC guidelines (Class II indication) (43% patients in group I vs. 70% in group II,  $p<0.05$ , Table 2). It was observed that such simultaneous administration of drugs was minimal in the Guardwire arm as compared to the other arm. Further, no major periprocedural adverse events including death, new Q wave or non Q wave AMI, emergent CABG, or repeat target vessel revascularization within 3 days of the index procedure were observed in the PercuSurge arm. In group I, one patient had Re MI and died in hospital while death occurred in one patient within 1 month of the PCI in group II.



a

2 (a) Figure shows LAD proximal segment 100 % blocked TIMI flow as well as TMP grade was 0 before PCI



b

(b) Figure shows the inflated distal balloon of PercuSurge device

Figure 2 continued on next page

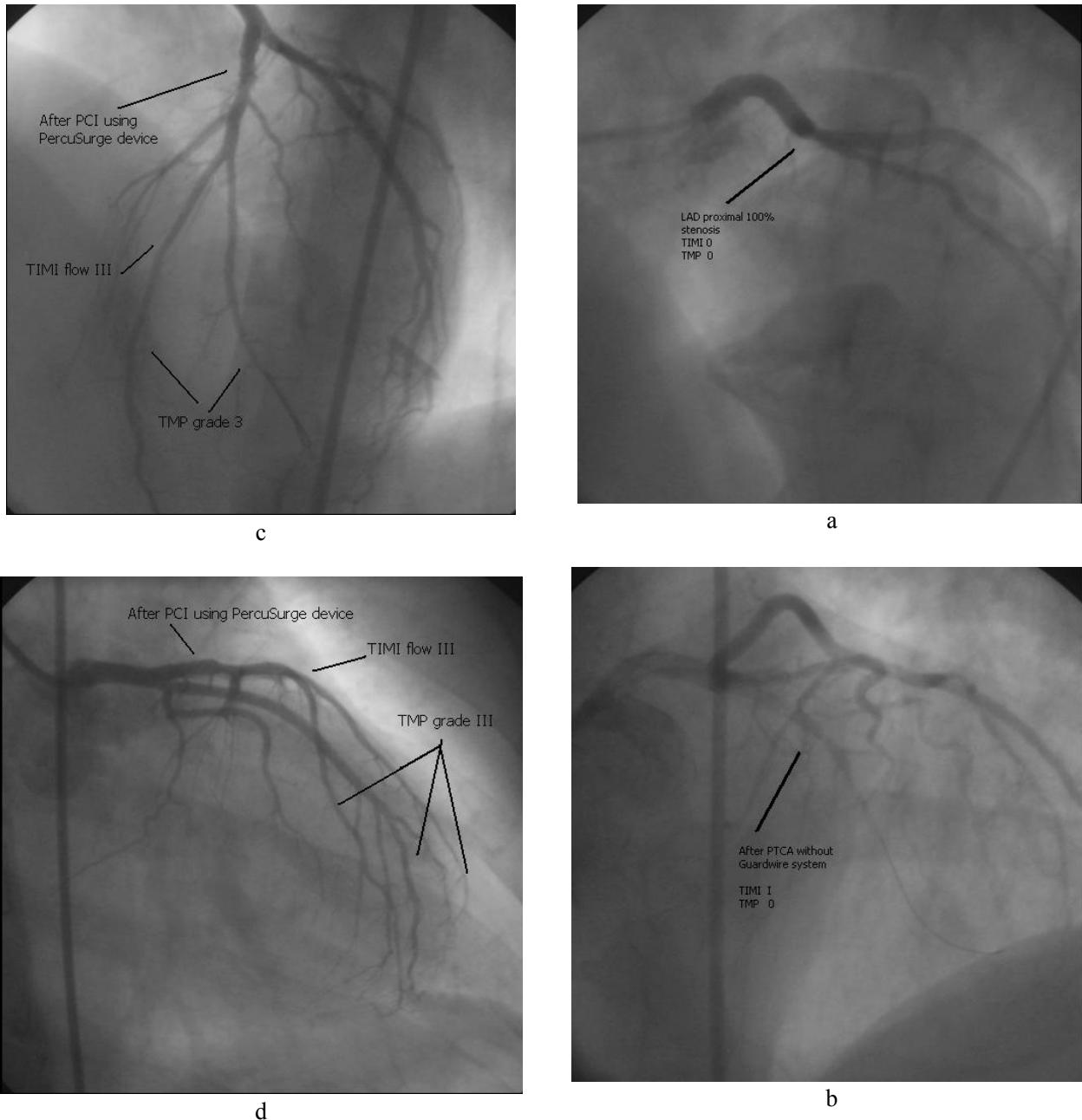


Figure 2: Angiograms of a patient treated with PCI using PercuSurge Guardwire system.  
(c) and (d) LAD Proximal segment shown in fig 2(a) treated with PCI with use of PercuSurge  
“No reflow” did not occur and the final TIMI flow III and TMP grade 3 was achieved. Figures 2(c) and 1(d) depict blood flow and myocardial blush

Figure 3: Angiogram of a patient treated with PCI without using PercuSurge Guardwire system. (a) Figure shows LAD proximal segment 100 % blocked TIMI flow as well as TMP grade was 0 before PCI  
(b) LAD Proximal segment in fig 1(a) treated with PCI without use of PercuSurge  
“No reflow” occurred and the final TIMI flow achieved was 1 and TMP grade was not improved

### Time in Minutes Involved in Different Steps of the Procedure

The total procedural time as well as time involved in individual steps of the PCI were recorded and compared (Table 3). It was observed that the time consumed in crossing the lesion by the wire was similar in both the groups ( $2.84 \pm 0.65$  minutes in group I vs.  $2.89 \pm 0.38$  minutes in group II). An additional time of  $3.04 \pm 0.57$  minutes was consumed in the PercuSurge arm to inflate the distal balloon. Inspite of this, the time consumed to reach PTCA balloon inflation after the wire crossing was identical in both the arms. Similarly, the total fluoroscopy time was also identical in both the groups (Table 3). The time to reach stent placement after wire crossing was significantly less ( $p<0.05$ ) in group I ( $9.26 \pm 1.25$  minutes) as compared to that in group II ( $14.55 \pm 2.27$  minutes).

We also calculated the time required after lesion crossing by wire till achievement of TIMI III / optimal TIMI flow (in case of PCI without use of PercuSurge).

This was found to be significantly less in Guardwire system group ( $21.04 \pm 2.19$  minutes) as compared to group II ( $28.58 \pm 2.52$  minutes,  $p=0.017$ ). The time frame from PTCA balloon inflation/stent placement (in case of direct stenting) till attainment of TIMI III / optimal TIMI flow was also significantly less in procedures with Guardwire use ( $17.37 \pm 2.21$  minutes in group I vs.  $24.41 \pm 2.5$  minutes in group II,  $p=0.025$ ). During this time frame the periprocedural adverse events like no reflow and distal embolization take place. The total procedural time consumed in PCI was found to be significantly less with use of PercuSurge as compared to PCI without its use ( $25.01 \pm 2.17$  minutes in group I vs.  $31.98 \pm 2.52$  minutes in group II,  $p=0.027$ ). The longest procedure in the PercuSurge group lasted 53.9 minutes where the shortest procedure was of 8.3 minutes whereas in group II, where PercuSurge was not used, the longest PCI lasted 69.1 minutes and the shortest procedure lasted 11.9 minutes.

**Table 3: Time involved in the different stages of the PTCA procedure**

Parameters	Group I (DPD) (n=30) Time in minutes	Group II (without DPD) (n=37) Time in minutes	p value
<i>Individual time frame for each step of the Procedure</i>			
Time for lesion crossing by wire	$2.84 \pm 0.65$	$2.89 \pm 0.38$	0.947
Time to inflate Distal balloon of Guardwire System	$3.04 \pm 0.57$	-	
<i>Total Time required for various steps of the procedure</i>			
Total time after wire crossing until PTCA balloon inflation	$4.4 \pm 0.67$	$4.17 \pm 0.61$	0.779
Total time after wire crossing until stent placement	$9.26 \pm 1.25$	$14.55 \pm 2.27$	0.037 *
<i>Total time after wire crossing to achievement of TIMI III flow</i>	$21.04 \pm 2.19$	$28.58 \pm 2.52$	0.017*
<i>Total time from PTCA balloon inflation (in POB) OR final stent Placement to TIMI III flow</i>	$17.37 \pm 2.21$	$24.41 \pm 2.5$	0.025*
Total Fluoroscopy time	$10.07 \pm 1.01$	$10.78 \pm 0.68$	0.507
Total PROCEDURAL TIME	$25.01 \pm 2.17$	$31.98 \pm 2.52$	0.027 *

Data expressed as Mean (time in minutes)  $\pm$  SEM

\* Unpaired Student's t test, Significantly less as compared to group II,  $p<0.05$

Group I: PCI with use of PercuSurge device

Group II: PCI without use of PercuSurge device

## Discussion

Microembolization may be a relatively frequent event among patients with acute coronary syndromes or after PCI. The benefit of primary PCI is limited by a 5% to 20% incidence of no reflow [28, 29]. The ‘no reflow’ phenomenon has been documented in ≥ 30% of patients after thrombolysis or PCI for AMI [4, 30], characterized by profound reduction in epicardial anterograde coronary flow without evidence of vessel dissection, thrombosis, or embolization.

Mechanical devices have recently emerged as an attractive tool to prevent both embolization in the microvasculature and no reflow [15, 20, 31]. Muller et al.[18] have shown that the PercuSurge system is a safe and effective device for the protection of distal embolization during interventions in degenerated aorto-coronary saphenous vein grafts. The study showed that the TIMI flow was significantly improved by the use of PercuSurge Guardwire system. Our study also shows that the number of patients achieving TIMI III flow was significantly higher with the use of PercuSurge as compared to PCI without its use. Even achievement of TMP III was significantly greater in patients with use of PercuSurge whereas PCI without its use could not result in optimal TIMI and TMP flow in all the cases. *The % of diabetics was significantly higher in group II (41%) as compared to group I (20%). This may be one of the factors responsible for the comparatively poorer outcome in group II. However, there are several other reports that support the contention that the use of PercuSurge is beneficial in preventing no-reflow as well as embolization in the distal microvasculature.* Huang et al.[24] have reported that no patient developed angiographic evidence of no reflow or distal embolization with the use of PercuSurge. In our study, also, we observed that the incidences of no/slow reflow were significantly less with use of PercuSurge as compared to PCI without its use, irrespective of the indications. Our study also showed that the use of PercuSurge significantly reduced the requirement of GP IIb/IIIa inhibitors and intracoronary administration of vasodilators like adenosine, sodium nitroprusside, etc. that were employed to overcome the no/slow reflow and distal embolization in the thrombotic lesions. In our study myocardial blush grade 3 was achieved in 85% of

patients, which is similar to that shown by Huang et al., i.e. 86.7% of patients.

Preliminary encouraging results with PercuSurge have been confirmed in the SAFER (Saphenous Vein Graft Angioplasty Free of emboli randomized) trial [20]. A significant reduction in MACE, MI and no reflow was reported in degenerated SV grafts. SAFE study<sup>19</sup> has also proved the efficacy of PercuSurge in patients with degenerated SVGs. However, very few studies have been done in AMI involving native coronaries. The preliminary results of RUBY (Revascularization Utilizing Balloon protection in acute coronary ischemic syndrome) registry have shown the efficacy of this Guardwire in the setting of AMI. The interim conclusions of RUBY registry suggest a high number of patients with TIMI III and low 30-day mortality in AMI patients using PercuSurge for distal protection. Our study also supports the above results along with a significant finding of less time consumed in the procedure.

The results of the single, large scale, Guardwire trial in AMI patients, EMERALD (Enhanced Myocardial Efficacy and Recovery by Aspiration of Liberated Debris) have been recently released. It has shown that the Guardwire was not associated with an improvement in the primary endpoints of post procedure ST resolution or infarct size. Also, the procedure duration was significantly longer in the Guardwire arm in this trial. Contrary to these results of EMERALD study, our results show that the total procedural time is in fact significantly less with the use of Guardwire system. In our trial, however, we have considered the precise period after the guide catheter engagement till the last cinefilm as the end of procedure to determine the total procedural time. This stringent time slot consideration may be responsible for our results, contrary to the EMERALD trial.

Pershad et al [32] has, in fact, in a case, reported thrombus at the site of the distal occlusive balloon of PercuSurge. However, no large-scale data is available in the line of this finding. In fact, in our study no procedural complication was observed. The device success and procedural success achieved were 100%. We also report for the first time that the total time required after lesion crossing by wire till achievement of TIMI III was significantly less as compared to PCI without its use. This time represents the slot when the atherothrombotic plaque is ruptured leading to distal

micro-embolization and no reflow, thus complicating the procedure. Various agents including GP IIb/IIIa inhibitors and intracoronary vasodilators like adenosine, sodium nitroprusside; NTG, etc. are thus employed to overcome this complication that then prolongs the time of PCI if PercuSurge is not used. We found in our study that using PercuSurge provides distal protection against microembolization and aspiration of the thrombotic debris, thus reducing the incidences of no reflow. Even the total procedural time was significantly less in PCI using the PercuSurge system by experienced operators.

### *Significance and Limitations of Our Study*

Unlike the EMERALD trial, our study did show lesser time consumed to achieve TIMI III flow using distal protection device and also better TIMI flow and TMP grade. This single centered study is limited by small size and the lack of a randomized set up. However, this will certainly be one of the important aspects for any future study using this protective device in native coronary arteries.

Our experience shows that at least 10 cases using the PercuSurge device have to be done before the operator and nurses develop proficiency in its use. The speed of the procedure is related to the "learning curve". It is our opinion that centers with higher usage of Guardwire system takes lesser time, which has a direct and clinically relevant impact on the outcome.

### **Conclusions**

These preliminary results suggest the efficacy and time saving aspect of the PercuSurge Guardwire system for distal protection against embolization during urgent coronary intervention in AMI. It is effective in reducing the risk of intraprocedural embolization and no reflow phenomenon without increasing the procedural time. These findings are contrary to the large, multi-centered EMERALD trial. We suggest that interventional cardiologists get experience with distal protection devices, which are efficient and time saving.

### **References**

- [1] Keeley EC, Boura JA, Grines CL. Primary angioplasty vs. intravenous thrombolytic therapy for acute myocardial infarction: a quantitative review of 23 randomized trials. *Lancet* 2003; 361: 13-20.
- [2] Topol EJ, Yadav JS. Recognition of the importance of embolization in atherosclerotic vascular disease. *Circ* 2000; 101:570-80.
- [3] Prati F, Pawlowski T, Gil R, Labellarte A, Gziut A, Caradonna E, Manzoli A, Pappalardo A, Burzotta F, Boccanfelli A. Stenting of culprit lesions in unstable angina leads to marked reduction in plaque burden: a major role of plaque embolization? A serial intravascular ultrasound study. *Circ* 2003; 107:2320-25.
- [4] Ito H, Maruyama A, Iwakura K, Takiuchi S, Masuyama T, Hori M, Higashino Y, Fujii K, Minamino T. Clinical implications of "no reflow" phenomenon: a predictor of complications and left ventricular remodeling in reperfused anterior wall myocardial infarction. *Circ* 1996; 93: 223-8.
- [5] Saber R.S, Edwards WD, Bailey KR, McGovern TW, Schwartz RS, Holmes DR Jr. Coronary embolization after balloon angioplasty or thrombolytic therapy: an autopsy study of 32 cases. *J Amer Coll Cardiol* 1993; 22: 1283-8.
- [6] Yip HK, Wu CJ, Chang HW, Chen MC, Hang CL, Fang CY, Hsieh YK, Yang CH, Yeh KH, Fu M. Comparison of impact of primary percutaneous transluminal coronary angioplasty and primary stenting on short term mortality in patients with cardiogenic shock and evaluation of prognostic determinants. *Amer J Cardiol* 2001; 87: 1184-8.
- [7] Yip HK, Chen MC, Chang HW, Hang CL, Hsieh YK, Fang CY, Wu CJ, Yang CH. Angiographic morphologic features of infarct-related arteries and timely reperfusion in acute myocardial infarction: predictors of slow-flow and no-reflow. *Chest* 2002; 122: 1322-32.
- [8] Tanaka A, Kawarabayashi T, Nishibori Y, Sano T, Nishida Y, Fukuda D, Shimada K, Yoshikawa J. No-reflow phenomenon and lesion morphology in patients with acute myocardial infarction. *Circ* 2002; 105: 2148-52.
- [9] Gibson CM, de Lemos JA, Murphy SA, Marble SJ, McCable CH, Cannon P, Antman EM, Braunwald E. Combination therapy with abciximab reduces angiographically evident thrombus in acute myocardial infarction: a TIMI 14 substudy. *Circ* 2001; 103: 2550-4.
- [10] Singh M, Berger PB, Ting HH, Rihal CS, Wilson SH, Lennon RJ, Reeder GS, Bresnahan JF, Holmes DR Jr. Influence of coronary thrombus on outcome of percutaneous coronary angioplasty in the current era (the Mayo Clinic experience). *Amer J Cardiol* 2001; 88: 1091-96.
- [11] Macdonald RG, Feldman RL, Conti CR, Pepine CJ. Thromboembolic complications of coronary angioplasty. *Amer J Cardiol* 1984; 54:916-7.

- [12] Weyne AE, H3eyndrickx GR, Vandekerckhove YR, Clement DL. Embolization of complicating coronary angioplasty in the presence of an intracoronary thrombus. *Clin Cardiol* 1986; 9:463-5.
- [13] Cameron J, Buchbinder M, Wexler L, Oesterle SN. Thromboembolic complications of percutaneous transluminal coronary angioplasty for myocardial infarction. *Cathet Cardiovasc Diagn* 1987; 13:100-6.
- [14] Arora RR, Platko WP, Bhadwar K, Simpfendorfer C. Role of intracoronary thrombus in acute complications during percutaneous transluminal coronary angioplasty. *Cathet Cardiovasc Diagn* 1989; 16:226-9.
- [15] Grube E, Gerckens U, Yeung AC, Rowold S, Kirchhof N, Sedgewick J, Yadav JS, Stertzer S. Prevention of distal embolization during coronary angioplasty in saphenous vein grafts and native vessels using porous filter protection. *Circ* 2001; 104:2436-41.
- [16] Van Ommeren V, Michels R, Heymen E, Van Asseldonk J, Bonnier H, Nainer J, De Swart H, Koolen J. Usefulness of the rescue PT catheter to remove fresh thrombus from coronary arteries and bypass grafts in acute myocardial infarction. *Amer J Cardiol* 2001; 88: 306-8.
- [17] Beran G, Lang I, Schreiber W, Denk S, Stefenelli T, Syeda B, Maurer G, Glogar D, Siostrzonek P. Intracoronary thrombectomy with the X-sizer catheter system improves epicardial flow and accelerates ST segment resolution in patients with acute coronary syndrome: a prospective, randomized, controlled study. *Circ* 2002; 105: 2355-60.
- [18] Muller R, Gerckens U, Staberock M, Grube E. Clinical experiences with the PercuSurge Guardwire: a new system for prevention of peripheral embolisms in catheter interventions on degenerated coronary artery venous bypasses. *Z Kardiol* 2000; 89: 316-22.
- [19] Grube E, Schofer JJ, Webb J, Schuler G, Colombo A, Sievert H, Gerckens U, Stone GW, Saphenous Venous Graft angioplasty free of emboli (SAFE) trial study group. Evaluation of a balloon occlusion and aspiration system for protection from distal embolization during stenting in saphenous vein grafts. *Amer J Cardiol* 2002; 89: 941-945.
- [20] Baim DS, Wahr D, George B. on behalf of the Saphenous vein graft angioplasty free of Emboli Randomized (SAFER) trial investigators. Randomized trial of a distal embolic protection device during percutaneous intervention of saphenous vein aorto-coronary grafts. *Circ* 2002; 105: 1285-90.
- [21] Henry M, Amor M, Henry I, Klonaris C, Chati Z, Masson I, Kownator S, Luizy F, Hugel M. Carotid stenting with cerebral protection: first clinical experience using the PercuSurge Guardwire system. *J Endovasc Surg* 1999; 6: 321-31.
- [22] Al-Mubarak N, Roubin GS, Vitek JJ, Lyer SS, New G, Leon MB. Effect of the distal balloon protection system on microembolization during carotid stenting. *Circ* 2001; 104: 1999-2002.
- [23] Henry M, Henry I, Klonaris C, Masson I, Hugel M, Tzvetanov K, Ethevenot G, Le BE, Kownator S, Luizi F, Folliguet B. Benefits of cerebral protection during carotid stenting with the PercuSurge Guardwire system: midterm results. *J Endovasc Ther* 2002; 9: 1-13.
- [24] Huang Z, Katoh O, Nakamura S, Negoro S, Kobayashi T, Tanigawa J. Evaluation of the PercuSurge Guardwire Plus Temporary Occlusion and aspiration system during primary angioplasty in acute myocardial infarction. *Cathet Cardiovasc Interv* 2003; 60: 443-51.
- [25] Sutsch G, Kiowski W, Bossard A, Luscher TF, Maier W, Vogt P, Amann FW. Use of an emboli containment and retrieval system during Percutaneous coronary angioplasty in native coronary arteries. *Schweiz Med Wochenschr* 2000; 130:1135-45.
- [26] Gibson MC, Cannon CP, Murphy SA, Ryan KA, Mesley R, Marble SJ, McCabe CH, Van de Werf F, Braunwald E for the TIMI study group. *Circ* 2000; 101: 125-30.
- [27] Van't Hof AWJ, Liem A, Suryapranata H, Hoornij JCA, Jan de Boer M, Zijlstra F. Angiographic assessment of myocardial reperfusion in patients treated with primary angioplasty for acute myocardial infarction. *Circ* 1998; 97: 2302-6.
- [28] Cura FA, L'Allier PL, Kapadia SR, Houghtaling PL, Dipaola LM, Ellis SG, Topol EJ, Brener SJ. Predictors and prognosis of suboptimal coronary blood flow after primary coronary angioplasty in patients with acute myocardial infarction. *Amer J Cardiol* 2001; 88: 124-8.
- [29] Yip HK, Wu CJ, Chang HW, Fang CY, Yang CH, Chen SM, Hung WC, Chen CJ, Cheng CI, Hsieh YK. Effect of the PercuSurge Guardwire Device on the Integrity of Microvascular and clinical outcomes during primary transradial coronary intervention in acute myocardial infarction. *Amer J Cardiol* 2003; 92: 1331-5.
- [30] Eeckhout E, Kern MJ. The coronary no-reflow phenomenon: a review of mechanisms and therapies *Eur Heart J* 2001; 22: 729-39.
- [31] Belli G, Pezzano A, De Biase AM, Baonacina E, Silva P, Salvade P, Piccalo G, Klugmann S. Adjunctive thrombus aspiration and mechanical protection from distal embolization in primary percutaneous intervention for acute myocardial infarction. *Cathet Cardiovasc Interv* 2000; 50:362-70.
- [32] Pershad A, Cherukiri G, Kirby A. PercuSurge guardwire balloon associated thrombus—a limitation of the PercuSurge distal protection system. *J Invasive Cardiol* 2002; 630-2.





## ARTICLE

# The Effect of an Alpha-Linolenic-Acid-Rich Diet on the Circadian Rhythm of Cardiac Events

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## Abstract

### *Objective*

Coronary artery disease events have a circadian rhythmicity, clustering more in the second quartile of the day. n-3 fatty acid supplementation reduces the rate of cardiac events, but its effect on their circadian rhythmicity has not been tested.

### *Design*

The Indo-Mediterranean Diet Heart Study was a single blind randomized study that assessed the effect of a diet rich in alpha-linolenic acid, the parent n-3 fatty acid, on the occurrence of myocardial infarction and sudden cardiac death.

### *Subjects*

One thousand subjects of the Indo-Mediterranean Diet Heart Study, focusing on the 115 patients from both control and intervention groups in which cardiac events occurred.

### *Intervention*

The timing of cardiac events throughout the day was compared between the intervention and control groups. The distribution of cardiac events along the four quartiles of the day was compared between groups as well as against equal distribution.

### *Results*

The risk ratio for a cardiac event was lowest between 4:00 and 8:00 in the morning for the intervention group. The control group had a higher,

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rate of events in the second quartile of the day, which deviated from an equal distribution, as expected ( $P=0.013$ ). In the intervention group events were equally distributed along the day. No statistically significant difference was found in daily event distribution between the groups.

### **Conclusion**

A diet rich in alpha-linolenic acid may abolish the higher rate of cardiac events, normally seen in the second quartile of the day. Additional studies are needed to identify the underlying mechanism.

## **Introduction**

The classical manifestations of coronary artery disease (CAD), such as angina pectoris, silent ischemia, myocardial infarction (MI) and sudden cardiac death, have a pronounced circadian rhythmicity, tending to cluster in the second quartile of the day [1-10]. This increase in the rate of cardiovascular events in the morning may be due to a hypercoagulable state, consisted of increased platelet aggregation, increased tPA inhibitor levels, and rapid metabolism of heparin in the morning [11-13]. In addition, the morning hours are associated with a decrease in vagal tone and increase in sympathetic activity, resulting in lower levels of acetylcysteine and melatonin and increased concentrations of cortisol, aldosterone, catecholamines and angiotensin, which make the atherosclerotic plaques more vulnerable to rupture and thrombosis [14-16]. These circadian manifestations are associated with a decreased heart rate variability (HRV), which is an independent risk factor for cardiovascular events [13-16].

Recent studies indicate that n-3 fatty acids may have a beneficial effect on HRV, platelet aggregation, and endothelial function, and that they are neuroprotectors and may enhance hippocampal acetylcysteine levels [15-26]. In randomized controlled trials, treatment with n-3 fatty acids led to a significant reduction in cardiac events, in both patients after a MI and those with risk factors only [27-30]. To our knowledge, no study has examined the effect of n-3 fatty acids on the timing of cardiovascular events. The aim of the present study is to examine the influence of a diet rich in alpha-

linolenic acid, the parent n-3 fatty acid, on the circadian rhythm of cardiac events.

## **Methods**

### *Subjects and Study Design*

The Indo-Mediterranean Diet Heart Study was a randomized, single-blind study designed to assess the effect of a diet rich in n-3 fatty acids from plant sources on the occurrence of myocardial infarction and sudden cardiac death [29]. One thousand subjects were randomized to either an intervention or a control group by selection of a card from a pile. The intervention group consisted of 499 patients, and the control group 501. The ethics committee of the Medical Hospital and Research Centre at Moradabad approved the study, and written informed consent was obtained from all participants.

Participants in both groups were advised to eat food elements that would provide a dietary intake similar to that recommended at the time by the National Cholesterol Education Program (NCEP) step I prudent diet [31], wherein less than 30% of energy comes from total fat, less than 10% from saturated fat, and less than 300 mg of cholesterol is consumed per day. Patients in the intervention group were advised to verify the intake of at least 250–300 g of fruit, 125–150 g of vegetables, and 25–50 g of nuts. They were also encouraged to eat 400–500 g of whole grains (legumes, rice, maize, wheat) daily, as well as to use mustard seed or soy bean oil in 3 to 4 servings per day, in order to ensure a high intake of alpha-linolenic acid.

Both groups were advised to exercise daily and practice yoga meditation techniques, to abstain from smoking and alcohol consumption, and to continue the use of their regular medications.

Food intake and physical activity were monitored by self-report diaries, first weekly, then monthly, and then every 3 months. Nutrient intakes were calculated using Indian foods composition tables and other sources [32,33] and reinforce adherence. Height, weight, and blood pressure were monitored by standard methods. Blood concentrations of glucose, total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol

and triglycerides were also measured. Clinical data, drug intake, adverse events, blood pressure, blood glucose levels, blood lipid levels, hospital admission and coronary events were recorded by physicians blinded to patient grouping.

At the 2-year follow up in the original study, cardiac events had occurred in 39 subjects from the intervention group and in 76 subjects from the control group.

### *Cardiac Events*

The principal endpoints of the original study were fatal or nonfatal MI, sudden cardiac deaths, and the combined total of these events. Owing to the relatively small number of events in each category, we did not attempt a subgroup analysis and focused only on the combined end-point of total cardiac events.

In hospitalized patients, a diagnosis of nonfatal MI was based on a diagnostic electrocardiograph at the time of the event, or the presence of ischaemic cardiac pain and diagnostic enzyme levels of at least twice the upper limit of normal, or the presence of ischaemic cardiac pain and equivocal enzyme levels and an equivocal electrocardiograph. In patients who had not been admitted to the hospital, the diagnosis of MI was considered if they complained of chest pain, breathlessness, or syncope, combined with a diagnostic electrocardiograph at the time of event or new electrocardiographic changes consistent with MI at routine check-up. The electrocardiographic coding and analysis were performed by a cardiologist blinded to patient grouping.

Fatal MI was diagnosed when a hospital record was consistent with cause of death, and there was either a preterminal hospital admission with a definite MI, or official documentation of a myocardial infarction.

Sudden cardiac death was diagnosed when coronary heart disease had been noted, and death occurred within one hour of symptom onset.

### *Statistical Analysis*

Analyses were performed in two ways. One was determining the risk for cardiac events in specific time frames among the whole study population. Second, we determined the number of events in the four quartiles of the day among only those subjects with events in both groups.

In order to assess the effect of the ALA-rich diet on the timing of cardiac event, we constructed a Cox multivariate model, with cardiac event as outcome variable, stratified into six 4-hour periods (0:01-4:00, 4:01-8:00, 8:01-12:00, 12:01-16:00, 16:01-20:00, and 20:01-24:00). In each model, we examined the risk ratio associated with the intervention diet, with patient's age, gender and BMI as covariates. The last available values served as the final risk factors data for the subjects who had fatal events. The analyses were held using standard statistical package (SPSS 11.0).

When comparing only the subgroups in which events occurred, Student's *t* test with a Bonferroni adjustment was used to analyze between-group differences of the continuous variables; P values less than 0.005 were considered statistically significant. The distribution of the number of events in each quartile of the day was compared by the chi-square test. Event rates were compared between the control and intervention groups, as well as for each group against equal distribution. Thus, for the latter, the observed event rates in the control group were compared with an expected rate of 19 (76 divided by 4) events in each quartile of the day, and the observed events in the intervention group were compared with an expected rate of 9.75 (39 divided by 4) in each quartile of the day. A two-tailed P value less than 0.05 was considered statistically significant for the parametric variables.

## **Results**

### *Timing of Events in Total Study Population*

Baseline characteristics of the Indo-Mediterranean Diet Heart study population was presented elsewhere [29]. In all strata, the ALA-rich diet was associated with protective effect for cardiac

events. The lowest risk ratio (RR=0.05; 95%CI: 0.01-0.24) was calculated for cardiac events between 4:01 and 8:00, while the highest was for events between 0:01 to 4:00 (RR=0.15 (95% CI:0.03 – 0.87) as shown in Figure 1. However, differences between strata did not reach statistical significance.

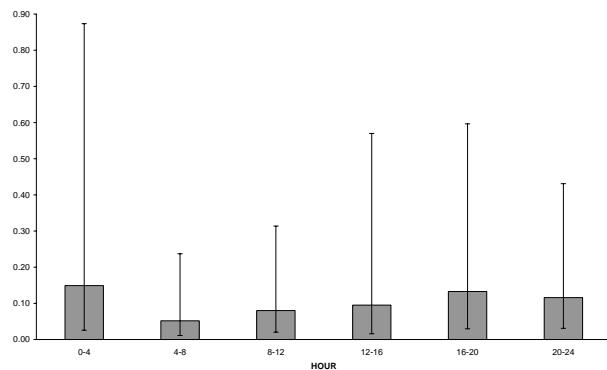


Figure 1. The risk ratio for cardiac events in the intervention group (n=499) compared with the control group (n=501) distributed into 4-hour intervals of the day.

#### *Dispersion of Events Along Quartiles of the Day*

Baseline characteristics of the subjects with cardiac events from both groups is presented in Table

1. Subjects in both groups were comparable for all major baseline variables except for body weight (borderline significance,  $P=0.006$ ), yet BMIs were comparable between groups. At 2 years, only TC and LDL levels differed significantly between groups, with lower values noted in the intervention group. Nutritional data are presented in Table 2. At baseline, values of all nutrients, including fatty acids, were similar in the two groups. At 2 years, n-3 fatty acid intake was significantly higher in the intervention group, and the n-6/n-3 fatty acid ratio was lower. The number of cardiovascular events of each type in both groups is given in Table 3.

The dispersion of cardiac events along the quartiles of the day is presented in Table 4. In the control group, there was an increased rate of cardiac events clustered in the second quartile of the day, which deviated from an equal distribution ( $P=0.013$ ). In the intervention group, the events were distributed equally along the day ( $P=0.49$  when comparing observed rates and equal distribution). There was no statistically significant difference in the rate of events in each quartile of the day between the two groups ( $P=0.54$ ).

**Table 1. Cardiovascular risk factors at baseline and after 2 years in the intervention and control groups. Data presented as means  $\pm$  SD. BMI- body mass index, BP- blood pressure, TC- total cholesterol, TG- triglyceride, FBG- fasting blood glucose.**

	Intervention Group A, n = 39			Control Group B, n = 76			P value at 2 years between groups
	Baseline	2 years	P value	Baseline	2 years	P value	
Age (years)	51 $\pm$ 10	-	-	49 $\pm$ 8	-	-	-
Weight, kg	65 $\pm$ 5	62 $\pm$ 7	0.0002	68 $\pm$ 6	67 $\pm$ 6	0.0004	<0.0001
BMI, kg/m <sup>2</sup>	24 $\pm$ 3	23 $\pm$ 3	0.0005	25 $\pm$ 2	24 $\pm$ 2	0.0005	0.0172
Systolic BP	134 $\pm$ 15	129 $\pm$ 15	0.0064	133 $\pm$ 19	132 $\pm$ 17	0.0040	0.2051
Diastolic BP	86 $\pm$ 6	84 $\pm$ 10	0.0931	88 $\pm$ 10	87 $\pm$ 10	0.0063	0.0532
Cigarettes / day	18 $\pm$ 9	19 $\pm$ 9	0.0961	21 $\pm$ 8	16 $\pm$ 5	0.0001	0.1608
TC, mmol/L	232 $\pm$ 43	220 $\pm$ 39	0.0029	245 $\pm$ 34	245 $\pm$ 32	0.7507	0.0001
LDL, mmol/L	150 $\pm$ 26	137 $\pm$ 30	0.0001	156 $\pm$ 32	157 $\pm$ 31	0.9745	0.0005
HDL, mmol/L	45 $\pm$ 15	44 $\pm$ 11	0.1319	44 $\pm$ 6	43 $\pm$ 11	0.3524	0.4414
TG, mmol/L	181 $\pm$ 51	169 $\pm$ 43	0.0259	176 $\pm$ 33	175 $\pm$ 35	0.8080	0.1881
FBG, mmol/L	112 $\pm$ 28	106 $\pm$ 25	0.0001	115 $\pm$ 33	109 $\pm$ 27	0.0029	0.2661

**Table 2.** Nutrient data at baseline and after 2 years in the intervention and control groups. Data presented as means  $\pm$  SD.

	Intervention Group A, n = 39			Control Group B, n = 76			P value at 2 years between groups
	Baseline	2 years	P value	Baseline	2 years	P value	0.2589
Energy (kcal.day)	2116 $\pm$ 175	2098 $\pm$ 241	0.3279	2167 $\pm$ 183	2123 $\pm$ 163	0.0099	<0.0001
CHO (%en)	57 $\pm$ 2	58 $\pm$ 3	0.0007	56 $\pm$ 2	55 $\pm$ 1	<0.0001	<0.0001
Protein (%en)	14 $\pm$ 1.7	14 $\pm$ 1.3	0.0112	14 $\pm$ 1.5	15 $\pm$ 0.8	0.1862	0.0241
Fat (%en)	27 $\pm$ 3	28 $\pm$ 3	0.0206	28 $\pm$ 2	30 $\pm$ 1.5	<0.0001	<0.0001
Saturated fat (%en)	13 $\pm$ 1.8	11 $\pm$ 2.3	0.0017	12 $\pm$ 1.4	13 $\pm$ 1.6	0.0003	<0.0001
MUFA (%en)	7.3 $\pm$ 1.6	9.0 $\pm$ 2.2	<0.0001	8.1 $\pm$ 2.1	8.8 $\pm$ 1.3	0.3857	0.01929
PUFA (%en)	7.0 $\pm$ 1.0	7.5 $\pm$ 1.0	<0.0001	7.5 $\pm$ 1.0	7.9 $\pm$ 0.8	0.0111	0.0052
n-6 (%en)	6.8 $\pm$ 0.9	6.9 $\pm$ 1.0	0.4042	7.3 $\pm$ 1.0	7.6 $\pm$ 0.8	0.0001	<0.0001
n-3 (%en)	0.2 $\pm$ 0.1	0.5 $\pm$ 0.2	<0.0001	0.2 $\pm$ 0.1	0.3 $\pm$ 0.1	0.0003	<0.0001

**Table 3.** Distribution of cardiac events in intervention and control groups. MI – myocardial infarction.

	Intervention group (n=39)	Control group (n=76)
Nonfatal MI	21	43
Fatal MI	12	17
Sudden cardiac death	6	16

**Table 4.** Distribution of cardiac events along the quartiles of the day in the intervention and control groups. In the intervention group, events are near-equally distributed along the day. In the control group, quartile 2 has more events than would be expected assuming equal distribution.

Quartile range)	(time	Intervention group (n=39)	Control group (n=76)
	n (% within group)	n (% within group)	
1 (00:01 – 06:00)	10 (26%)	15 (20%)	
2 (06:01 – 12:00)	11 (28%)	29 (38%)	
3 (12:01 – 18:00)	8 (21%)	10 (13%)	
4 (18:01 – 00:00)	10 (26%)	22 (29%)	

## Discussion

This study is the first to assess the effect of an ALA-rich diet on the circadian rhythm of cardiac events. We have found that although the intervention diet dramatically lowered the risk for events throughout the day, it nearly abolished it in the early morning hours. The increased rate of events known to occur in the second quartile of the day was eliminated by the diet.

Although n-3-rich diets have been found to be cardioprotective in several randomized controlled trials [27-30], their exact mode of action is still unknown. Our results suggest that dietary supplementation of alpha-linolenic acid may lead to a change in the circadian occurrence of cardiac events. The only major risk factors that differed significantly between the groups at the end of the trial along the trial were the lower levels of total cholesterol and LDL. However, we would expect this change to influence the total rate of events, and not just their distribution during the day, which was the focus of this study.

We are unaware of any other large-scale study that has examined the effect of n-3 fatty acids on the circadian rhythmicity of cardiac events. The Physicians Health Study recorded the number of myocardial infarctions in patients treated with aspirin or not, and found a significant decrease in the

rhythmicity of myocardial infarctions in the aspirin group [34]. It is possible that this reduction was the result of the inhibitory effect of aspirin on platelet aggregation, a quality also possessed by n-3 fatty acids [25]. When these findings are combined, it appears that one of the triggers for MI in the morning hours is the increased tendency of platelets to aggregate.

Christensen and coworkers [17] reported a positive correlation between HRV and the platelet content of n-3 fatty acids (docosahexaenoic acid, DHA) in patients with post-MI left ventricular dysfunction. The increase in HRV occurred even after intake of only one fish meal per week, with an increase in standard deviation of normal-normal (SDNN) from 103 to 122ms. Further analysis revealed a negative correlation between the ratio of arachidonic acid/ DHA and the SDNN interval [18], consistent with earlier findings. In a more recent study, of 291 patients referred for coronary angiography that completed a food frequency questionnaire, researchers measured the n-3 fatty acid composition of granulocyte membranes and adipose tissue along with 24-hour HRV analysis [19]. Significant positive correlations were found between HRV indices and the levels of n-3 fatty acids in granulocytes. The positive influence of n-3 fatty acids on HRV suggests that this is indeed a major mechanism of CAD event reduction, as seen even after a few months of treatment [28].

HRV is a manifestation of sympathetic and parasympathetic activity of the heart, and a decreased HRV enhances the risk of circadian rhythmicity of cardiac events. This may well be another mechanism by which n-3 fatty acids reduce coronary events in the morning hours. In one randomized, controlled trial, of 81 patients after MI [17], treatment with n-3 fatty acids was associated with significant increase in HRV compared with controls, indicating an increased vagal cardiac tone. A retrospective analysis of a randomized, controlled intervention trial among 118 (fish oil group) and 122 (control group) patients with acute MI showed that treatment with fish oil (rich in EPA and DHA) was associated with a significant reduction in cardiac events (30% vs 56%,  $P<0.02$ ) [27]. While the majority of cardiac events in the control group were reported in the second quartile of the day compared to first and third quartiles (35.7%

vs 16.0%, 19.6%, respectively,  $P<0.01$ ), no such association was observed in the fish oil group (unpublished data). These results are consistent with the view that fish oil administration may have a beneficial effect on circadian rhythmicity of cardiac events.

Alpha-linolenic acid is the parent n-3 fatty acid which is converted into long-chain fatty acids such as EPA and DHA in the body, to be later incorporated among phospholipids in cell membranes and adipose tissue. By using a control group which was also given dietary and exercise advice, we were able to isolate and highlight the role of the n-3 fatty acids. A detailed analysis of the role of specific nutrients in our Indo-Mediterranean diet is currently underway. Interim calculations show that the dietary intake of n-3 fatty acids had a major impact on the risk for cardiac events in the intervention group. The main sources of n-3 fatty acids in our diet were plants: walnuts, green leafy vegetables, whole grains, soy bean oil, and mustard oil. Thus, it is not necessary to consume the fish-derived long chain acids, DHA and EPA, in order to reap the benefits of n-3 fatty acids.

The exact mechanism underlying the circadian rhythmicity of cardiac events and its regulation by n-3 fatty acids remains unknown. There is consistent evidence that this rhythmicity is controlled by the suprachiasmatic nucleus [35], a tiny clump of cells in the brain which works as a biological clock and is influenced by daily changes in light and darkness. Brain neurons are rich in n-3 fatty acids, as are cell membranes of cardiomyocytes, endothelial cells and arterial smooth muscle cells. It is therefore possible that a relative deficiency of n-3 fatty acids in these cells enhances their susceptibility to excitation, causing greater neurohumoral and cardiovascular dysfunction, and resulting in a marked increase in the rhythmicity of cardiac events [36]. Increased vagal tone decreases the synthesis of tumor necrosis factor-alpha (TNF-alpha) in the liver and inhibits the release of proinflammatory cytokines such as IL-6 [20-22]. It is possible that low levels of n-3 fatty acids decrease the vagal tone, leading to increased synthesis of TNF-alpha and a greater release of proinflammatory cytokines by increased sympathetic activity. Treatment with n-3 fatty acids may break this cycle, decreasing inflammation and excitation of neurons, cardiomyocytes, endothelial and smooth muscle cells,

bringing about a decreased vulnerability for cardiac events. Furthermore, n-3 fatty acids may also enhance brain acetylcysteine level and parasympathetic tone, resulting in increased HRV, and hence, protection from pronounced rhythmicity of cardiac events [17-22].

One major limitation of our study is the relatively small number of events both overall and in each quartile of the day, owing to both the concomitant primary prevention arm of our original study (about half of the 1000 subjects had only risk factors for CAD, and were not post-MI patients), and the large reduction in event rate in the intervention group, brought about by the n-3 supplementation. Thus, the statistical analyses cannot definitely rule out this factor as the reason for the fairly equal distribution of events in the n-3 group. Given the measured proportion of events in the high risk hours (28%), a power of 80% and 39 participants in the intervention group, allowed the minimal detection of 17% difference compared to the control group; the difference observed was 10%. Nevertheless, the many mechanisms by which n-3 fatty acids may influence the circadian rhythm of CAD events cannot be overlooked, and we therefore believe that a biological explanation is plausible. A larger interventional study with more events is needed to confirm our findings.

In summary, our study suggests that an Indo-Mediterranean diet, which is rich in fruits, vegetables, nuts, whole grains, soy and mustard oils, can provide sufficient alpha-linolenic acid, to result in a modulation in the rhythmicity of cardiac events by n-3 fatty acids. Since a high n-6/n-3 ratio may blunt the beneficial effects of n-3 fatty acids, soybean oil should be replaced by more mustard oil. The exact nature of the cardioprotective action of n-3 fatty acids, whether by influencing platelet function or HRV through brain-heart connections, remains to be elucidated.

## References

- [1] Pell S, D'Alonzo CA. Acute myocardial infarction in a large industrial population. *JAMA* 1963;185:831-8.
- [2] Arntz HR, Willich SN, Schreiber C, et al. Diurnal, weekly and seasonal variation of sudden death. *Eur Heart J* 2000;21:315-20.
- [3] Willich SN. European survey on circadian variation of angina pectoris (ESCV): design and preliminary results. *J Cardiovasc Pharmacol* 1999;34(suppl 2):s9-13.
- [4] Singh RB, Pella D, Neki NS, et al. Mechanism of acute myocardial infarction study (MAMY Study). *Biomed Pharmacother* 2004;57:in press.
- [5] Rocco MB, Barry J, Campbell BAS, et al. Circadian variation of transient myocardial ischemia in patients with coronary artery disease. *Circulation* 1987;75:395-400.
- [6] Muller JE, Stone PH, Turi ZG, et al. Circadian variation in the frequency of onset of acute myocardial infarction. *N Engl J Med* 1985;313:1315-22.
- [7] Goldberg R, Brady P, Chen J, et al. Time of onset of acute myocardial infarction after awakening. *J Am Coll Cardiol* 1989;13:133A [abstract].
- [8] Davies MJ, Thomas A. Thrombosis and acute coronary artery lesions in sudden cardiac ischemic death. *N Engl J Med* 1984;310:1137-41.
- [9] Muller JE, Ludmer PL, Willich SN, et al. Circadian variation in the frequency of sudden cardiac death. *Circulation* 1987;75:131-8.
- [10] International Study of Infarct Survival-2 (ISIS-2) Collaborative Group. Morning peak in the incidence of myocardial infarction: experience in the ISIS-2 trial. *Eur Heart J* 1992;13:594-608.
- [11] Toffler GH, Brezinski D, Schaefer A, et al. Concurrent morning increase in platelet aggregability and the risk of myocardial infarction and sudden cardiac death. *N Engl J Med* 1987;316:1514-8.
- [12] Chasen C, Muller JE. Cardiovascular triggers and morning events. *Blood Pressure Monit* 1998;3:35-42.
- [13] Deedwania PC. Hemodynamic changes as triggers of cardiovascular events. *Cardiol Clin* 1996;14:229-38.
- [14] Singh RB, Cornelissen G, Siegelova J, et al. About half-weekly (circaseptan) pattern of blood pressure and heart rate in men and women of India. *Scripta Medica* (Brno) 2002;75:125-8.
- [15] Singh RB, Weydahl A, Otsuka K, et al. Can nutrition influence circadian rhythm and heart rate variability? *Biomed Pharmacother* 2001;55 (suppl 11):s115-24.
- [16] Singh RB, Niaz MA, Cornelissen GS, et al. Circadian rhythmicity of circulating vitamin concentrations. *Scripta Medica* (Brno) 2001;74:93-6.
- [17] Christensen JH, Gustenhoff P, Komp E, et al. Effect of fish oil on heart rate variability in survivors of myocardial infarction. *Br Med J* 1996;312:677-8.
- [18] Christensen JH, Komp E, Aaroe J, et al. Fish consumption, n-3 fatty acids in cell membranes and heart rate variability in survivors of myocardial infarction with left ventricular dysfunction. *Am J Cardiol* 1997;79:1670-2.
- [19] Christensen JH, Skou HA, Fog L, et al. Marine n-3 fatty acids, wine intake, and heart rate variability in patients

- referred for coronary angiography. *Circulation* 2001;103:651-7.
- [20] Chin JPF. Marine oils and cardiovascular reactivity. *Prostaglandins Leukot Essent Fatty Acids* 1994;50:211-22.
- [21] Borovikova LV, Ivanova S, Zhang M, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 2000;405:458-62.
- [22] Das UN. The brain-lipid-heart connection. *Nutrition* 2001;17:260-3.
- [23] Lauritzen I, Blondeau N, Heurteaux C, et al. Polyunsaturated fatty acids are potent neuroprotectors. *EMBO J* 2000;19:1784-93.
- [24] Minami M, Kimura S, Endo T, et al. Dietary docosahexaenoic acid increases cerebral acetylcholine levels and improves passive avoidance performance in stroke-prone spontaneously hypertensive rats. *Pharmacol Biochem Behav* 1997;58:1123-9.
- [25] Goodnight SH Jr, Harris WS, Connor WE. The effects of dietary omega-3 fatty acids upon platelet composition and function in man: a prospective controlled study. *Blood* 1981;58:880-5.
- [26] Pella D, Singh RB, Otsuka K, et al. Nutritional predictors and modulators of insulin resistance. *J Nutr Environ Med* 2004;14:3-16
- [27] Singh RB, Niaz MA, Sharma JP, et al. Randomized, double blind, placebo controlled trial of fish oil and mustard oil in patients with suspected acute myocardial infarction. The Indian Experiment of Infarct Survival. *Cardiovasc Drug Ther* 1997;11:485-91.
- [28] Marchioli R, Barzi F, Bobma E, et al. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction. time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. *Circulation* 2002;105:1897-903.
- [29] Singh RB, Dubnov G, Niaz MA, et al. Effect of an Indo-Mediterranean diet on progression of coronary artery disease in high risk patients (Indo-Mediterranean Diet Heart Study): a randomised single-blind trial. *Lancet* 2002;360:1455-61.
- [30] de Lorgeril M, Salen P, Martin JL, et al. Mediterranean diet, traditional risk factors and the rate of cardiovascular complications after myocardial infarction. Final report of the Lyon Diet Heart Study. *Circulation* 1999; 99: 779-85.
- [31] National Cholesterol Education Program. Second report of the expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel II). *Circulation* 1994;89:1333-445.
- [32] Narasinga Rao BS, Deosthale YG, Pant KC. *Nutrient composition of Indian foods*. Hyderabad, India: National Institute of Nutrition, Indian Council of Medical Research. 1989.
- [33] Goodhart RS, Shils ME, eds. *Modern nutrition in health and disease*. Philadelphia, PA: Lea & Febiger, 1980.
- [34] Ridker PM, Manson JE, Buring JE, et al. Circadian variation of acute myocardial infarction and the effect of low dose aspirin in a randomized trial of physicians. *Circulation* 1990;82:897-902.
- [35] Ralph MR, Foster RG, Davis FC, et al. Transplanted suprachiasmatic nucleus determines circadian period. *Science* 1990;247:975-8.
- [36] Yehuda S. Omega-6/omega-3 ratio and brain related functions. *World Rev Nutr Diet* 2003;92:37-56.



## ARTICLE

# Myocardium Viability Assessed by Delayed Contrast-Enhanced Magnetic Resonance Imaging in Patients with Severe Ischemic Heart Failure: A Comparison with Thallium SPECT and Dobutamine Echocardiography

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## Abstract

### Purpose

Functional recovery of ischemic cardiomyopathy depends on accurate identification of viable myocardium. We evaluated the value of delayed contrast-enhanced magnetic resonance imaging (MRI) in the assessment of myocardial viability.

### Materials and Methods

Forty patients with significant coronary artery disease and severe left ventricular (LV) dysfunction received delayed contrast-enhanced MRI and <sup>201</sup>Tl single-photon emission computed tomography (SPECT) within one month. Ten of them received simultaneous <sup>201</sup>Tl SPECT and dobutamine stress echocardiography (DSE). Three imaging modalities were compared based on a standardized 17-segment model. Improved regional wall thickening on follow-up MRI was used as an index of functional recovery in patients received revascularization.

### Results

Of 680 segments, MRI hyperenhancement showed positive correlation with <sup>201</sup>Tl reduction ( $r = 0.61$ ,  $p < 0.0001$ ). The viability concordance was 75.3% between DSE and MRI, 80.5% between <sup>201</sup>Tl SPECT and MRI, and 66.5% among all tests. Contrast-enhanced MRI detected more myocardial viability than <sup>201</sup>Tl SPECT, especially in the inferior wall and apex ( $p < 0.0001$ ). Eight patients underwent revascularization. Comparing with functional recovery in the follow-up results, the diagnostic accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of delayed contrast-enhanced MRI were 64%, 95%, 24%, 62% and 77%, respectively. For <sup>201</sup>Tl SPECT, these

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values were 55%, 75%, 29%, 58% and 54%, respectively. Areas under the ROC curves by MRI and  $^{201}\text{TL}$  SPECT were  $0.59 \pm 0.04$ ,  $0.52 \pm 0.05$ , respectively ( $p=0.07$ ). Of 16 segments viable on MRI but nonviable on  $^{201}\text{TL}$  SPECT, 12 (75%) recovered function after revascularization, while only one of 3 segments classified as nonviable on MRI but viable on  $^{201}\text{TL}$  SPECT recovered function ( $p < 0.05$ ). Of 10 segments classified as scar by both tests, none recovered function after revascularization.

## Conclusion

Delayed contrast-enhanced MRI detects viability better than  $^{201}\text{TL}$  SPECT, especially in the inferior wall and apex. Myocardial viability detected by MRI may predict functional recovery after revascularization in ischemic cardiomyopathy, even in patients with severe LV dysfunction.

**Key Words:** myocardial viability, magnetic resonance imaging,  $^{201}\text{TL}$  SPECT, dobutamine stress, echocardiography

## Introduction

Improved ejection fraction (EF) after revascularization shows positive impact on prognosis in chronic ischemic cardiomyopathy [1,2]. Measurement of regional wall thickening of dysfunctional myocardium offers quantitative assessment of functional recovery after revascularization [3-5]. Determination of the viability of dysfunctional myocardium, however, remains a problem. The sensitivity of the dobutamine stress echo (DSE) is poor. Although the positron-emitting and single photon-emitting scintigraphic imaging techniques are known to assess myocardial viability, these modalities display rather lower specificity [1,2,5-8]. Cardiac MRI was reported to offer reliable and accurate assessment of myocardial scar burden [9], coronary perfusion [10] and contractile reserve [11]. The technique of delayed contrast-enhanced MRI using an inversion-recovery prepared  $T_1$ -weighted gradient-echo sequence was shown to provide exquisite contrast and spatial resolution of myocardial fibrosis [12,13]. While the infarct areas in the myocardium appeared cold regions on  $^{201}\text{TL}$  single-photon emission tomography ( $^{201}\text{TL}$  SPECT) [12], it was vividly enhanced on MRI [13]. In

addition, superior resolution of MRI made it more sensitive than other techniques to differentiate transmural from non-transmural infarction [14]. Larger transmural extent of contrast enhancement was found to correlate with less functional recovery after revascularization of dysfunctional myocardium [15]. To date, reports on comparing cardiac MRI,  $^{201}\text{TL}$  SPECT and DSE in the assessment of chronic ischemic cardiomyopathy are limited. The present study aimed to compare the delayed contrast-enhanced MRI with  $^{201}\text{TL}$  SPECT and DSE in the assessment of myocardial viability, and to evaluate its predictive power of functional recovery after revascularization in patients with chronic ischemic heart failure.

## Materials and Methods

### Patients

Between February, 2002 and September, 2004, forty consecutive patients (36 male; age,  $59.4 \pm 12.5$  years) with angiographically significant coronary artery disease (CAD) ( $\geq 70\%$  diameter stenosis) and symptoms of heart failure (New York Heart Association functional class  $\geq II$ ) for more than three months were prospectively enrolled for the evaluation of myocardial viability as shown in Table 1. All patients had a LVEF  $\leq 40\%$  and dysfunctional myocardium identified by resting echocardiography. All patients were excluded for recent myocardial infarction (MI) or unstable angina pectoris for  $<$  six weeks, valvular disease or contraindications to MRI as criteria laid down elsewhere. All patients consented under the approval of the Institutional Review Board of the National Taiwan University Hospital. All the imaging studies on myocardial viability received by each patient were performed within the duration of one month. Eight patients underwent revascularization within one month after viability study, and another cardiac MRI study was followed at least 3 months after the intervention. A flow chart of viability study and treatment of these patients is shown in the Figure 1.

**Table 1. Patient Demographics**

	Patients	N = 40
Male gender	36	90 %
Age, yrs	$59.4 \pm 12.5$	37 – 83
Risk factors		
Hypertension	25	63%
Diabetes	14	35%
Hyperlipidemia	14	35%
History of MI (> 6 weeks)	20	50%
Previous interventions	23	58%
PCI	20	
CABG	3	
NYHA functional class		
II	20	50%
III	14	35%
IV	6	15%
Mean	$2.7 \pm 0.7$	
CAD		
1-VD	4	10%
2-VD	12	30%
3-VD	24	60%

Values are mean  $\pm$  SD. MI = myocardial infarction; PCI = percutaneous coronary intervention; CABG = coronary artery bypass surgery; NYHA = New York Heart Association; CAD = coronary artery disease; VD = vessel-disease

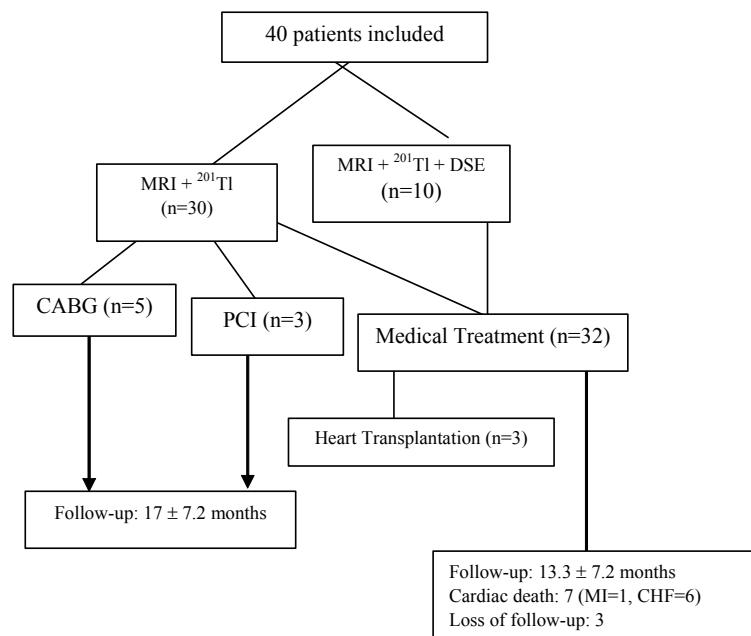


Figure 1. The flow chart of viability studies and treatments of patients included in this study. (DSE = dobutamine stress echocardiography; PCI = percutaneous coronary intervention; CABG = coronary artery bypass surgery; MI = myocardial infarction; CHF = congestive heart failure)

## Echocardiography

M-mode and 2D echocardiography data were received at the echo lab using a Sonos-4500/5500 imaging system (Hewlett Packard, Palo Alto, CA) to assess contractile function and measure left atrial and LV dimensions according to standard criteria [16].

## $^{201}\text{Tl}$ SPECT Protocols

Eight patients were studied under the exercise stress test. For the rest who were unable to exercise adequately, we used the pharmacological stress test: dipyridamole for 14 patients and simultaneous DSE and  $^{201}\text{Tl}$  SPECT for the remaining 10 patients.

The exercise stress test was performed using a modified Bruce protocol [17]. The injection of  $^{201}\text{Tl}$  (2-3 mCi) was timed at peak exercise. After the injection, exercise was continued for another minute.

For the dipyridamole stress test, intravenous dipyridamole (0.56 mg/kg in 4 minutes) was infused to induce coronary hyperemia and  $^{201}\text{Tl}$  was injected 3 minutes after the completion of the infusion [18].

Simultaneous DSE and  $^{201}\text{Tl}$  imaging was performed as described previously [5, 19]. In brief, dobutamine was intravenously infused at rates of 5, 10, 20, 30 and 40  $\mu\text{g}/\text{kg}/\text{min}$  in 3-minute stages. Atropine was given if the patient's heart rate was lower than 85% of age-predicted maximum heart rate.  $^{201}\text{Tl}$  was injected one minute before the termination of dobutamine infusion.

Stress SPECT imaging was started within 5 minutes after the injection of  $^{201}\text{Tl}$  and the redistribution imaging was acquired 4 hours later. If images showed irreversible defects, additional 1 mCi  $^{201}\text{Tl}$  was injected and a third set of images was obtained 15 minutes after the reinjection.

## Rest-Redistribution $^{201}\text{Tl}$ SPECT ( $n=8$ )

Eight patients underwent rest-redistribution  $^{201}\text{Tl}$  SPECT imaging because of their severe heart failure. The  $^{201}\text{Tl}$  SPECT images were acquired 15 minutes and 4 hours after administration of 2-3 mCi  $^{201}\text{Tl}$ . The post-processing and reconstruction of  $^{201}\text{Tl}$  images was performed as described elsewhere [5, 17-20].

## Cardiac MRI

Electrocardiographic-gated MRI was performed on a 1.5-T scanner (Sonata, Siemens, Erlangen, Germany) using a phased-array coil (CP Body Array Flex, Siemens, Erlangen, Germany) attached with respiratory and cardiac gating during repeated breath holds. Using steady-state free precession, MRI cine images were acquired as multiple short-axis views (every 1 cm throughout the entire LV) and 2 perpendicular long-axis planes. In another session, gadolinium contrast (Gd-DTPA, 0.15 mmol/kg) was administered intravenously, and contrast-enhanced images were acquired 10 minutes after administration using a segmented inversion-recovery prepared turbo gradient echo technique [15] in the identical planes. The imaging parameters were as follows: TR/TE/flip angle = 750 ms/4.18 ms/25°; FOV/matrix size/spatial resolution = 290×340 mm/216×256/0.75 mm; and the inversion time (TI) = 250 ms optimized to null the signal intensities of normal myocardium.

## Image Processing and Analysis

The MRI images were interpreted by two independent blinded radiologists. If two interpretations did not agree with one another, the images were reviewed by the two physicians to reach a consensus. The LV myocardium was divided into 17 segments for analysis as AHA criteria recommendation [21].

## Echocardiographic Analysis

Segmental wall motion and contractile reserve were defined by echocardiography before and after low-dose infusion of dobutamine as described earlier with accuracy of interobserver agreement 94% ( $\kappa=0.85$ ) [17,22].

## SPECT Image Analysis

SPECT images were normalized to peak myocardial activity as 100%.  $^{201}\text{Tl}$  activity in each segment was scored as 0 = normal perfusion, 1 = mild

decrease of photon counts, 2 = moderate decrease of photon counts, 3 = severe decrease of photon counts, and 4 = absent photon counts [23]. Each segment in SPECT images was scored for viability by perfusion photon counts normalized to peak myocardial activity as 100% based on the methods used previously [23, 24]. Our interobserver agreement for  $^{201}\text{TI}$  SPECT interpretations was 96% as previously reported [17].

### *MR Image Analysis*

LV function parameters were assessed in a standard way using computing software (Matlab<sup>®</sup>, MathWorks Inc., MA, USA). The planimeter method was used to determine LV volumes, LVEF and cardiac mass and endocardial and epicardial borders on all short-axis cine images [25]. Wall thickening on cine MR images was measured as the percentage of systolic wall thickening (SWT) according to the following formula: (end-systolic wall thickness – end-diastolic wall thickness)/end-diastolic wall thickness  $\times 100\%$ . The delayed contrast enhancement (DC) in each segment was quantified using computing software (Mathematica<sup>®</sup>, Wolfram Research, Inc., IL, USA), following the criteria as described elsewhere [26]. The percentage of pixels showing hyperintensities out of the total myocardial pixels was determined by a 4-score grading: grade 0, no DC; grade 1, 1% to 25% DC; grade 2, 26% to 50% DC; grade 3, 51% to 75% DC; grade 4, > 75% DC. To compare MRI with SPECT and DSE data, grade 3 defined as non-viable myocardium. In the follow-up study, an increase of  $\geq 15\%$  SWT change was used to define improvement of post-revascularization contractile function [27].

### *Statistical Analysis*

Data were collected as mean  $\pm$  SD showing comparisons between groups by use of 2-sample *t* test and chi-square analysis for continuous and categorical variables, respectively. ANOVA (analysis of variance) with Bonferroni's post hoc test was performed to detect any associations between two or more variables. Agreement between the diagnostic tests was evaluated using kappa index. Correlations

between the extent of DC on MRI and  $^{201}\text{TI}$  uptake on SPECT were analyzed using linear regression analyses. Changes between baseline and follow-up measurements were assessed by paired *t* tests. ROC curves were used to compare predictive accuracy of both MRI and  $^{201}\text{TI}$  SPECT to predict an increase of  $\geq 15\%$  SWT change at follow-up. Areas under the ROC curves were compared by use of the z test. A *p* value  $< 0.05$  (two-sided) was predetermined to be statistically significant. All statistical analyses were done using scientific statistical software packages (Stata 8, Texas, USA).

## **Results**

Forty patients with demography as shown in Table 1, were studied at baseline by MRI and  $^{201}\text{TI}$  SPECT. Ten of them underwent simultaneous DSE and  $^{201}\text{TI}$  studies. Of these patients, eight underwent revascularization after viability studies, including percutaneous coronary intervention (PCI) in three (left anterior descending artery in two patients and left circumflex artery in one patient) and coronary bypass surgery (CABG) in five patients. Follow-up MRI studies were performed  $5.0 \pm 4.3$  months after the revascularization procedures. One patient had non-ST elevation myocardial infarction during PCI. No other patient had clinical events between two MRI studies.

Of patients who were treated medically, three received heart transplantation within one month after viability studies. Seven patients died during the follow-up periods, including acute MI in one and intractable heart failure in another six patients as shown in Figure 1.

### *Comparison of Viability Data*

The results of myocardial viability assessed by MRI and  $^{201}\text{TI}$  SPECT are shown in Table 2 (A, B). Viable myocardium detected on MRI had positive relationship with  $^{201}\text{TI}$  activity. The concordant rate of viability was 80.5% between  $^{201}\text{TI}$  SPECT and MRI ( $\kappa=0.52$ ), and overall concordant rate was 66.5% among DSE,  $^{201}\text{TI}$  SPECT and MRI tests ( $\kappa=0.51$ ).

The contractile reserve detected by late enhancement on MRI showed higher proportion of

transmural extent of DC on MRI (Table 2C). In some cases, contractile reserve was impaired resulting with invisible or minor scar detected on MRI (21/102, 21%). All segments with extensive scar on MRI and or SPECT showed no contractile function at rest (Figure 2), although almost all segments with > 50% DC showed marked impairment of resting function.

**Table 2. Comparison of Myocardial Viability Using Different Diagnostic Modalities**

**(A) Relationship between viability detected by dobutamine stress echocardiography, MRI and  $^{201}\text{TI}$  SPECT**

170 segments	DSE +		DSE -	
	TI +	TI -	TI +	TI -
MRI +	79	12	23	14
MRI -	3	2	3	34

**(B) Concordance between viability detected by MRI and  $^{201}\text{TI}$  SPECT**

680 segments	MRI	
	+	-
$\text{TI}^+$	411	33
	93	109

**(C) Relationship between transmural extent of DC on MRI and contractile reserve detected by dobutamine stress echocardiography**

170 segments	Transmural Extent of DC on MRI			
	0 - 25%	26- 50%	51- 75%	76-100%
DSE +	81	10	5	0
DSE -	21	17	17	9

DSE = dobutamine stress echocardiography;  $\text{TI} = {^{201}\text{TI}}$  SPECT; + = viable; - = scar, DC = delayed contrast enhancement

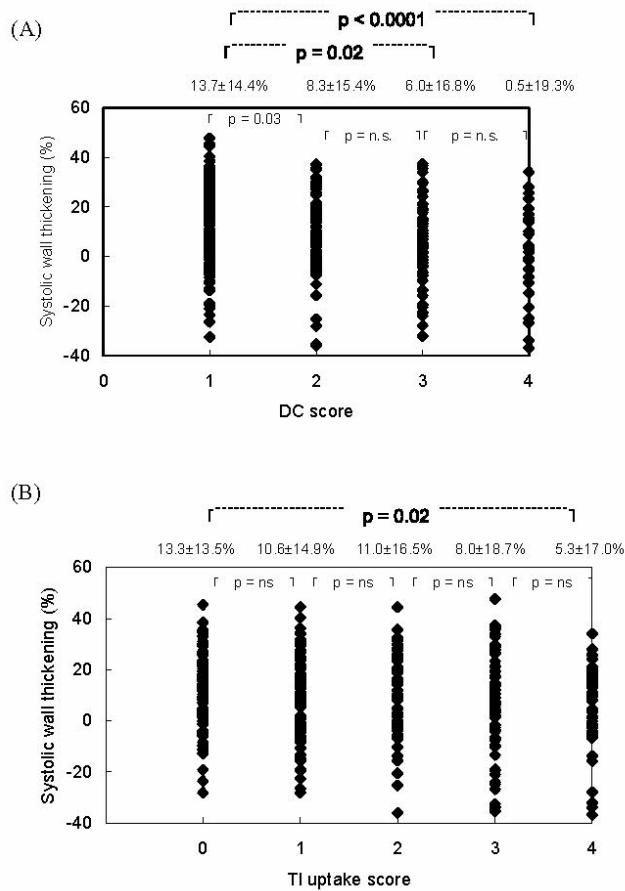


Figure 2. Relationship between (A) delayed contrast enhancement on MRI, (B)  $^{201}\text{TI}$  uptake on SPECT and percentage of systolic wall thickening on MRI (ns = non-significant, DC = delayed contrast enhancement)

In 680 segments from 40 patients, the amount of viable tissue on MRI correlated positively with  $^{201}\text{Tl}$  uptake ( $^{201}\text{Tl}$  uptake score = 0.67 DC score - 0.69,  $r=0.61$ ,  $p < 0.0001$ ). The extent of DC enhancement on MRI and the reduction of  $^{201}\text{Tl}$  uptake both showed agreement ( $\kappa=0.51$ , Figure 3). The MRI detected more myocardial viability than  $^{201}\text{Tl}$  SPECT in the inferior wall (92/120 vs. 63/120,  $p < 0.0001$ ), and apex (23/40 vs. 15/40,  $p < 0.0001$ ). The discrepancy between viability determined by  $^{201}\text{Tl}$  SPECT and MRI is shown in Table 3.

The representative examples of concordant and discordant myocardial viability detected by MRI and  $^{201}\text{Tl}$  SPECT are shown in Figure 4 and 5.

### Follow-Up Data

A total of 106 segments (97 dysfunctional) in eight patients were revascularized after viability tests. Among 97 dysfunctional segments, 84 (87%) were viable on MRI and 71 (73%) were viable on  $^{201}\text{Tl}$  SPECT. Two of the eight patients had evidence of new delayed enhanced segments in their post-revascularization scan (one patient in CABG group had three and the other patient in PCI group had two

new delayed enhanced segments). The LVEF in the eight patients at baseline was  $26.3 \pm 8.1\%$  and increased significantly to  $38.6 \pm 16.8\%$  at follow-up ( $p=0.04$ ). Total LV mass ( $217.5 \pm 54.5$  to  $183.0 \pm 47.9$  g), LV end-diastolic volume index ( $110.5 \pm 38.7$  to  $86.6 \pm 36.0$  mL/m $^2$ ), LV end-systolic volume index ( $82.3 \pm 37.4$  to  $55.9 \pm 36.9$  mL/m $^2$ ) were also decreased postoperatively, but statistically insignificant.

In terms of regional function change, the SWT measured by MRI was used as the parameter of myocardial viability. Of the 97 dysfunctional segments, 55 (57%) showed functional recovery (increase of segmental SWT  $\geq 15\%$ ). The overall diagnostic accuracy, sensitivity, specificity, positive predictive value and negative predictive value of viability detected by MRI were, respectively, 64%, 95%, 24%, 62% and 77%, whereas they were 55%, 75%, 29%, 58% and 54% by  $^{201}\text{Tl}$  SPECT. Moreover, the SWT improvement after revascularization in dysfunctional segments was dependent on its transmural extent shown in Table 4. However, there was no significant difference in areas under the ROC curves by MRI and  $^{201}\text{Tl}$  SPECT, respectively ( $0.59 \pm 0.04$  vs.  $0.52 \pm 0.05$ ,  $p=0.07$ ).

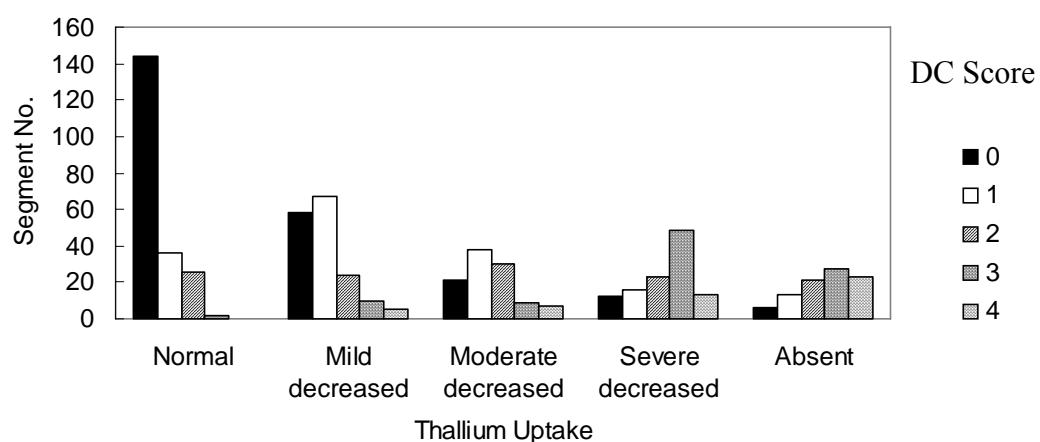


Figure 3. Relationship between thallium uptake and delayed contrast enhancement in MRI. (DC = delayed contrast enhancement)

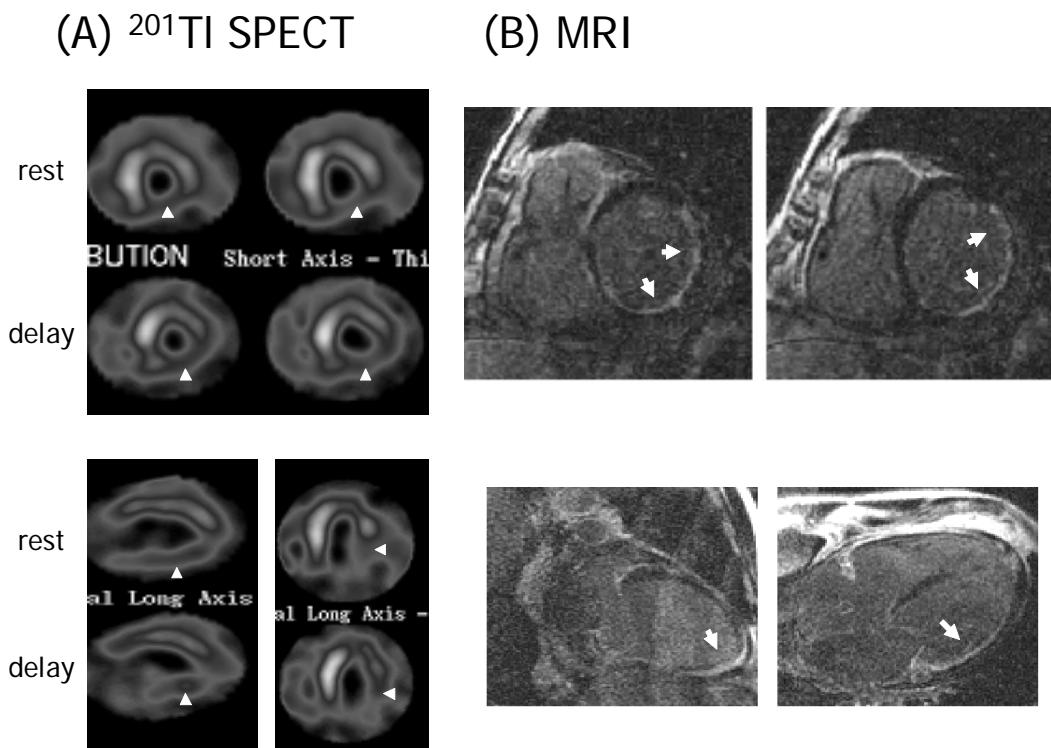


Figure 4. A 64-year-old patients with old inferior myocardial infarction (LVEF 17%) showed concordant scars in the inferior and inferolateral walls on the rest-redistribution  $^{201}\text{TI}$  SPECT (A, arrow heads) and delayed contrast-enhanced MRI (B, arrows)

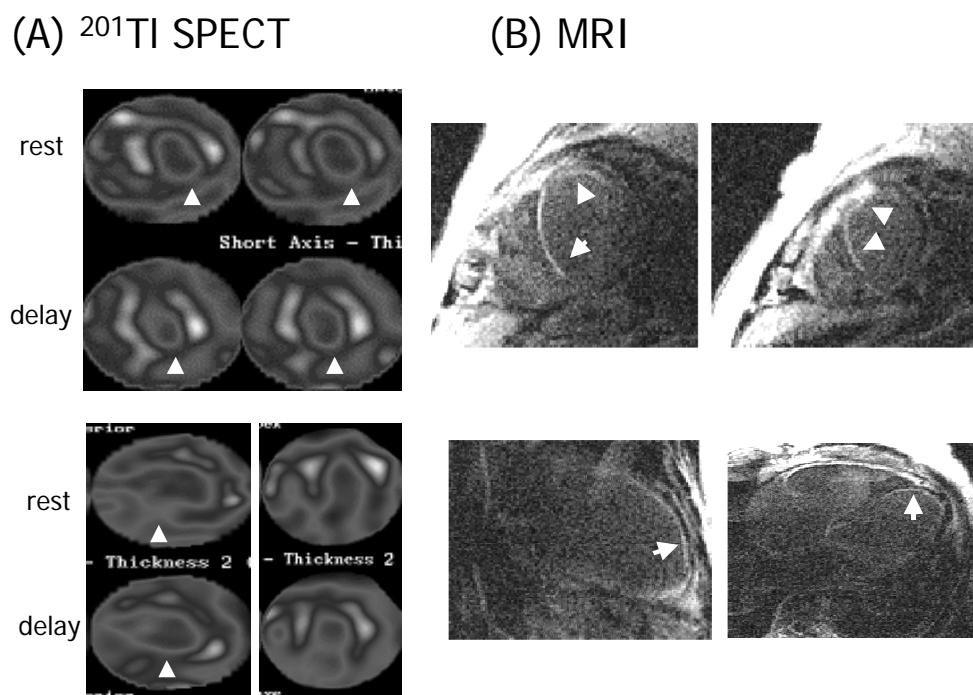


Figure 5. A 43-year-old man with 3-vessel disease (LVEF 8%) showed scar in the inferior wall (arrow heads) on the rest-redistribution  $^{201}\text{TI}$  SPECT (A), which is discordant with contrast-enhanced MRI (B) showing delayed enhancement in the anterior and septal walls (arrows)

**Table 3. Distribution of Discrepancies in Identifying Myocardial Viability by  $^{201}\text{TI}$  SPECT and MRI**

(A) Scar detected by MRI but viable by $^{201}\text{TI}$ SPECT (n = 33 segments)							
	Anterior	Anteroseptal	Inferoseptal	Inferior	Inferolateral	Lateral	Apex
Basal	1	1	4	0	0	2	2
Mid-ventricular	2	3	4	1	3	2	
Apical	1		4	2		2	

(B) Scar detected by $^{201}\text{TI}$ SPECT but viable by MRI (n = 93 segments)							
	Anterior	Anteroseptal	Inferoseptal	Inferior	Inferolateral	Lateral	Apex
Basal	3	4	5	10	9	0	9
Mid-ventricular	3	4	1	11	5	1	
Apical	6		6	11		5	

**Table 4. Influence of Transmural Extent of Delayed Contrast Enhancement on Recovery of Regional Function in 97 Dysfunctional Segments at Baseline**

Transmurality	Systolic Wall Thickening Ratio (%)				<i>P</i>
	Baseline	Follow-Up	Difference		
0 - 25% (n=52)	9.7 ± 14.8	28.0 ± 12.9	17.9 ± 15.6		< 0.0001
26 - 50% (n=32)	6.2 ± 13.9	15.2 ± 15.3	9.0 ± 18.6		0.01
51 - 75% (n=9)	0.1 ± 6.0	3.7 ± 19.0	3.6 ± 17.9		0.56
76 - 100% (n=4)	-11.9 ± 17.1%	-1.8 ± 22.8	10.2 ± 29.7		0.56

Of sixteen segments viable on MRI but nonviable on  $^{201}\text{TI}$  SPECT, twelve (75%) segments showed function recovery after revascularization, while only one of three segments classified as nonviable on MRI but viable on  $^{201}\text{TI}$  SPECT recovered function ( $p < 0.05$ ). Out of ten segments classified as scar by both tests, no segment showed the function improvement after revascularization. Out of five new segments with hyperenhancement after the interventions, four segments indicated unrecovered regional wall function with transmural involvement (SWT change,  $-0.19 \pm 8.9\%$ ). However, improvement was seen in the only segment with hyperenhancement confined to the subendocardial layer (SWT change, 33.75%).

## Discussion

The identification of hibernating myocardium in patients with chronic heart failure is becoming state of the art in cardiovascular science. Previous studies have shown great potentials in terms of outcome and recovery of LV function after revascularization [1-5]. Myocardial viability assessment by  $^{18}\text{F}$ -fluorodeoxyglucose ( $^{18}\text{F}$ -FDG) positron emission

tomography (PET) is clinically important in these patients, but high costs and limited availability of PET cameras and radiotracers hamper the clinical applications. Recent advance in cardiac MRI allows imaging myocardial viability with excellent imaging quality [9-15]. However, reports on its value in predicting functional recovery after revascularization in patients with chronic severe LV dysfunction, in comparison with well-established  $^{201}\text{TI}$  SPECT and DSE, are still lacking.

Our results are in agreement with previous works [28,29] that dobutamine response depends on the transmural scar extent shown on DC. Our data corroborated with previous reports [1,6-8,29] and highlighted that DSE detected less viable myocardium in comparison with both  $^{201}\text{TI}$  SPECT and MRI imaging methods. However, the concordance between DSE and MRI (75.3%) or between DSE and  $^{201}\text{TI}$  SPECT (76.5%) was similar.

In the present study, we observed that the amount of viable myocardial tissue on cardiac MRI was positively correlated with  $^{201}\text{TI}$  uptake. Similar findings were reported to assess by  $^{18}\text{F}$ -FDG PET and MRI techniques [27,30]. Our data were consistent with previous findings that MRI identified

subendocardial infarcts but missed by SPECT [14,29]. We also observed that  $^{201}\text{Tl}$  SPECT might overestimate the scar tissue, especially in the inferior wall and apex. These discrepancies may be explained in part by the low-energy X-ray emission and soft tissue attenuation. The inferior wall is one of the most sensitive to  $^{201}\text{Tl}$  attenuation region in the heart, thus greater attenuation of  $^{201}\text{Tl}$  in the inferior segments could be anticipated. However, limited success was reported by using infarct location adjusted thresholds for viability assessment [31-34]. The regions of dysfunctional myocardium seen as viable on  $^{201}\text{Tl}$  SPECT but MRI invisible can be attributed to the fact that  $^{201}\text{Tl}$  scan could become positive in the presence of a thin rim of viable tissue as previously reported [35,36].

Earlier reports showed that in patients with LV dysfunction undergoing revascularization, the extent and severity of nonviable tissue defined by MRI correlates well correlated with the likelihood of functional recovery assessed by LVEF [16,27]. Our study extends the information further showing relationship of chronic CAD and severe LV dysfunction in our research volunteers (most patients with LVEF  $\leq 25\%$ ). Our results further suggest that the myocardial viability detected by MRI may be valuable on the clinical evaluation of potential benefits from high-risk revascularization procedures.

The functional recovery assessment shown in our study after intervention was not homogeneous. Several factors affect the functional recovery including different intervention procedures, completeness of revascularization, and peri- and post-operative MI events [37,38]. Furthermore, the degree and duration of pre-procedural cellular structure change may influence the functional recovery.

However, there are some recognized limitations. Our sample size was relatively small, and limited patients underwent revascularization. Neither of the patients who received simultaneous DSE and  $^{201}\text{Tl}$  imaging received the revascularization, so the comparison between DSE and MRI in predicting functional recovery is still lacking. The  $^{201}\text{Tl}$  imaging method in this study reflected our routine clinical protocol, no additional correction algorithm or prone position imaging was applied, which has been proposed to decrease the false-positive rate [39,40].

## Conclusion

Cardiovascular MRI provides a unique tool to assess cardiac structure and function in a single study session. The present study demonstrates the good correlations among DSE,  $^{201}\text{Tl}$  SPECT and MRI in the detection of myocardial viability. MRI detects better viability than  $^{201}\text{Tl}$  SPECT, especially in the inferior wall and apex. MRI has marginally higher predictive value in predicting functional recovery. For patients with severe ischemic cardiomyopathy, noninvasive MRI may provide better clinical decision on using high-risk revascularization procedures.

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## References

- [1] Allman KC, Shaw LJ, Hachamovitch R, et al. Myocardial viability testing and impact of revascularization on prognosis in patients with coronary artery disease and left ventricular dysfunction: a meta-analysis. *J Am Coll Cardiol* 2002; 39:151-158.
- [2] Underwood SR, Bax JJ, vom Dahl J, et al. Imaging techniques for the assessment of myocardial hibernation: report of a study group of the European Society of Cardiology. *Eur Heart J* 2004; 25:815-836.
- [3] Rahimtoola SH. A perspective on the three large multicenter randomized clinical trials of coronary bypass surgery for chronic stable angina. *Circulation* 1985; 72(6 pt 2) V-123-V-135.
- [4] Van-den-Berg EK Jr, Popma JJ, Dehmer GJ, et al. Reversible segmental left ventricular dysfunction after coronary angioplasty. *Circulation* 1990; 81:1210-6.
- [5] Huang PJ, Lin LC, Yen RF, et al. Accuracy of biphasic response, sustained improvement and worsening during dobutamine echocardiography in predicting recovery of myocardial dysfunction after revascularization: comparison with simultaneous thallium-201 reinjection SPECT. *Ultrasound Med Biol* 2001; 27:925-931.
- [6] Shan K, Constatine G, Sivananthan M, Flamm S. Role of cardiac magnetic resonance imaging in the assessment of myocardial viability. *Circulation* 2004; 109:1328-1334.
- [7] Dilsizian V, Rocco TP, Freedman NMT, Leon MB, Bonow RO. Enhanced detection ischemic but viable

- myocardium by the reinjection of thallium after stress-redistribution imaging. *N Engl J Med* 1990; 323:141-146.
- [8] Cigarroa C, De Fillipi C, Brickner ME, Alvarez L, Wait MA, Grayburn PA. Dobutamine stress echocardiography identifies hibernating myocardium and predicts recovery of left ventricular function after coronary revascularization. *Circulation* 1993; 88:430-436.
- [9] Mahrholdt H, Wagner A, Holly TA, et al. Reproducibility of chronic infarct size measurement by contrast-enhanced magnetic resonance imaging. *Circulation* 2002; 39:1151-1158.
- [10] Al Saadi N, Nagel E, Gross M, et al. Noninvasive detection of myocardial ischemia perfusion reserve based on cardiovascular magnetic resonance. *Circulation* 2000; 101:1379-1383.
- [11] Baer FM, Theissen P, Schneider CA, et al. Dobutamine magnetic resonance imaging predicts contractile recovery of chronically dysfunctional myocardium after successful revascularization. *J Am Coll Cardiol* 1998; 31:1040-1048.
- [12] Ramani K, Judd RM, Holly TA, et al. Contrast magnetic resonance imaging in the assessment of myocardial viability in patients with stable coronary artery and left ventricular dysfunction. *Circulation* 1998; 98:2687-2694.
- [13] Kim RJ, Fieno DS, Parrish TB, et al. Relationship of MRI delayed contrast enhancement to irreversible injury, infarct age, and contractile function. *Circulation* 1999; 100:1992-2002.
- [14] Wagner A, Mahrholdt H, Regenfus M, et al. Contrast-enhanced MR and routine single photon emission computed tomography (SPECT) perfusion imaging for detection of subendocardial myocardial infarcts: an imaging study. *Lancet* 2003; 361:364-379.
- [15] Kim RJ, Wu E, Rafael A, et al. The use of contrast-enhanced magnetic resonance imaging to identify reversible myocardial dysfunction. *N Engl J Med* 2000; 343:1445-1453.
- [16] Oh JK, Seward JB, Tajik J. The echo manual. 2<sup>nd</sup> ed. Philadelphia, *Lippincott Williams and Wilkins*, 1999; 7-43.
- [17] Huang PJ, Chieng PU, Lee YT, et al. Exercise thallium-201 tomographic scintigraphy in the diagnosis of coronary artery disease: emphasis on the effect of exercise level. *J Formos Med Assoc* 1992; 91:1096-1101.
- [18] Ho FM, Huang PJ, Liau CS, Lee FK, Chieng PU, Su CT. Dobutamine stress echocardiography compared with dipyridamole thallium-201 single-photon emission computed tomography in detecting coronary artery disease. *Eur Heart J* 1995; 16:570-575.
- [19] Huang PJ, Ho YL, Wu CC, et al. Simultaneous dobutamine stress echocardiography and thallium-201 perfusion imaging for the detection of coronary artery disease. *Cardiology* 1997; 88:556-562.
- [20] Lomboy CT, Schulman DS, Grill HP, et al. Rest-redistribution thallium-201 scintigraphy to determine myocardial viability early after myocardial infarction. *J Am Coll Cardiol* 1995; 25:210-217.
- [21] Cerqueira MD, Weissman NJ, Dilsizian V, et al. Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart. *Circulation* 2002; 105:539-542.
- [22] American Society of Echocardiography Committee on Standards: Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. *J Am Soc Echocardiogr* 1989; 2:258-267.
- [23] Kiat H, Maddahi J, Roy LT, Van Train K, Friedman J, Resser K, et al. Comparison of technetium-99m methoxyisobutyltyrele and thallium-201 evaluation of coronary artery disease by planar and tomographic methods. *Am Heart J* 1989; 117:1-11.
- [24] Dilsizian V, Freedman NMT, Bacharach SL, Perrone-Filardi P, Bonow RO. Regional thallium uptake in irreversible defects: magnitude of change in thallium activity after reinjection distinguishes viable from nonviable myocardium. *Circulation* 1992; 85:627-634.
- [25] Tseng WY, Liao TY, Wang JL. Normal systolic and diastolic functions of the left ventricle and left atrium by cine magnetic resonance imaging. *J Cardiovasc Magn Reson* 2002; 4:443-457.
- [26] Mahrholdt H, Wagner A, Holly TA, et al. Reproducibility of chronic infarct size measurement by contrast-enhanced magnetic resonance imaging. *Circulation* 2002; 106:2322-2327.
- [27] Knuesel PR, Nanz D, Wyss C, et al. Characterization of dysfunctional myocardium by positron emission tomography and magnetic resonance: relation to functional outcome after revascularization. *Circulation* 2003; 108:1095-1100.
- [28] Shan K, Bick RJ, Poindexter BJ, et al. Altered adrenergic receptor density in myocardial hibernations in humans: a possible mechanism of depressed myocardial function. *J Am Coll Cardiol* 1996; 28:432-442.
- [29] Nelson C, McCrohon J, Khafagi F, et al. Impact of scar thickness on the assessment of viability using dobutamine echocardiography and thallium single photon emission computed tomography: a comparison with contrast-enhancement magnetic resonance imaging. *J Am Coll Cardiol* 2004; 43: 1248-1256.
- [30] Klein C, Nekolla SG, Bengel FM, et al. Assessment of myocardial viability with contrast-enhanced magnetic resonance imaging: comparison with positron emission tomography. *Circulation* 2002; 105:162-167.
- [31] Vanoverschelde J-LJ, DeHondt A-M, Marwick T, et al. Head-to-head comparison of exercise-redistribution-reinjection thallium single photon emission computed tomography and low dose dobutamine echocardiography for prediction of reversibility of chronic left ventricular ischemic dysfunction. *J Am Coll Cardiol* 1996; 28:432-442.
- [32] Gunnung MG, Anagnostopoulos C, Knight CJ, et al. Comparison of TI-201, technetium-99m tetrafosmin and dobutamine magnetic resonance imaging in identifying

- hibernating myocardium. *Circulation* 1998; 98:1869-1874.
- [33] Udelson JE, Coleman PS, Metherall J, et al. Predicting recovery of severe regional ventricular dysfunction. Comparison of resting scintigraphy with 201 Tl and 99m-Tc-sestamibi. *Circulation* 1994; 89:2552-2561.
- [34] Schneider CA, Voth E, Gawlich S, et al. Significance of rest technetium-99m sestamibi imaging for the prediction of improvement of left ventricular dysfunction after Q wave myocardial infarction: importance of infarct location adjusted thresholds. *J Am Coll Cardiol* 1998; 32:648-654.
- [35] Zamorano J, Delgado J, Almeria C, et al. Reason for discrepancies in identifying myocardial viability by thallium-201 redistribution, magnetic resonance imaging and dobutamine echocardiography. *Am J Cardiol* 2002; 90:455-459.
- [36] Baumgartner H, Porenta G, Lau YK, et al. Assessment of myocardial viability by dobutamine echocardiography, positron emission tomography and thallium-201 SPECT. Correlation with histopathology in explanted hearts. *J Am Coll Cardiol* 1998;32: 1701-1708.
- [37] Ricciardi MJ, Wu E, Davidson CJ, et al. Visualization of discrete microinfarction after percutaneous coronary intervention associated with mild creatine kinase-MB elevation. *Circulation* 2001; 103:2780-2783.
- [38] Selvanayagam JB, Petersen SE, Francis JM, et al. Effects of off-pump versus on-pump coronary surgery on reversible and irreversible myocardial injury. A randomized trial using cardiovascular magnetic resonance imaging and biochemical markers. *Circulation* 2004; 109:345-350.
- [39] Esquerre JP, Coca FJ, Martinez SJ, et al. Prone decubitus: a solution to inferior wall attenuation in thallium-201 myocardial tomography. *J Nucl Med* 1989; 30:398-401.
- [40] Hendel RC, Berman DS, Cullom SJ, et al. Multicenter clinical trial to evaluate the efficacy of correction for photon attenuation and scatter in SPECT myocardial perfusion imaging. *Circulation* 1999; 99:2742-2749.



## ARTICLE

# Beneficial Effects of Addition of Fenofibrate to Statin Therapy in Patients with Acute Coronary Syndrome after Percutaneous Coronary Interventions

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## Abstract

### Objective

The objective of the present investigation was to find out whether the addition of fenofibrate to statin monotherapy produced any synergistic or additive beneficial effects in reducing risk factors, especially plasma fibrinogen, in patients of Acute Coronary Syndromes (ACS) requiring Percutaneous Coronary Interventions (PCI).

### Methods

This was a randomized, non-blind, prospective study with parallel group design, conducted in 102 patients who had angiographically documented Coronary Artery Disease (CAD). All had undergone angioplasty. The patients were randomized to atorvastatin (20mg/day, n=25), simvastatin (40mg/day, n=27), atorvastatin(10mg/day)-fenofibrate (200mg/day) combination (n=25) and simvastatin (20mg/day)-fenofibrate(200mg/day) combination (n=25). The serum lipid profile and plasma fibrinogen were recorded before initiation of therapy and after 3 months of the respective treatments.

### Results

All the patients already had desirable lipid levels as per the NCEP ATP III guidelines. The addition of fenofibrate to statin monotherapy produced additional benefits on reduction in triglyceride (TG) and very low density lipoprotein (VLDL) levels, and an increase in high density lipoprotein (HDL) levels. All the treatment groups showed a significant decrease in the plasma fibrinogen levels. This did not correlate with any of the study parameters like age, body weight, hemodynamic characteristics and lipoprotein levels. Statin monotherapy produced a significant decrease in the fibrinogen levels and the addition of fenofibrate further enhanced the reduction.

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## Conclusions

Addition of fenofibrate to statins seems to be beneficial in patients with ACS. Statins, contrary to various reports, were found to decrease plasma fibrinogen significantly. Further, in combination with fenofibrate there was enhanced reduction of the novel risk factor, fibrinogen.

**Key words:** Acute coronary syndromes, statins, fenofibrate, plasma fibrinogen

## Introduction

The last century has seen a rapid increase in the global prevalence of Coronary artery disease (CAD). Estimates from the Global Burden of Disease Study have predicted that India faces the greatest health burden due to Coronary Artery Disease.[1] A number of inflammatory markers have been studied for their ability to predict future cardiovascular events in asymptomatic individuals and patients with established atherosclerotic disease.[2] Among the emerging novel cardiac markers, plasma fibrinogen has been identified as an important risk factor for cardiovascular diseases. Many cross-sectional, case controlled, and numerous prospective cohort studies have identified elevated plasma fibrinogen levels as an independent risk factor for coronary heart disease, stroke, and peripheral vascular disease [3]

Fibrinogen is an acute phase protein that is directly involved in coagulation. The transcription of fibrinogen is stimulated by IL-6; its synthesis is suppressed by IL-1 $\beta$  and TNF- $\alpha$ . [4,5] Fibrinogen and its metabolites strongly affect hemostasis, hemorheology, platelet aggregation and endothelial function. Infact, fibrinogen's association with increased mortality lies probably in its ability to promote thromboses, or clots, by causing platelet aggregation in blood vessels. The recognition that fibrinogen is an important factor in the promotion of various disease states has led to the search for specific therapies intended to reduce plasma fibrinogen levels.

The use of statins in the prevention of primary [6] and secondary [7,8] CAD has been demonstrated to significantly reduce cardiovascular events and total mortality. Nevertheless, the majority of patients on statin treatment still experience coronary events. [9] It

is clear that a more effective reduction in the incidences of coronary events is needed. This could probably be accomplished by a further reduction in the conventional as well as novel risk factors like plasma fibrinogen, homocysteine, and C-reactive protein. Multiple small studies have reported changes in fibrinogen levels with different statins. [10, 11] While atorvastatin is claimed to increase plasma fibrinogen, simvastatin is reported to either increase or have a neutral effect. Although many different pharmacologic approaches and strategies for therapeutic modulation of fibrinogen have been tested, the efficacy of different treatments to lower plasma fibrinogen in humans is limited and the mode of action unidentified. [12, 13] Among the few compounds known to lower circulating fibrinogen levels in humans are certain fibrates.

Fibrates reportedly lower plasma fibrinogen in humans, but the regulatory mechanism of this effect remains to be clarified. Activation of the nuclear hormone receptor PPAR $\alpha$  mediates the suppression of fibrinogen gene transcription by fibrates in rodents.[3] This establishes PPAR $\alpha$  as a key regulatory factor in fibrinogen gene expression in rodents and may explain the suppressive effect of fibrates on plasma fibrinogen levels in humans. The fibrinogen molecule is arranged as a dimer, with each monomer composed of 3 nonidentical polypeptide chains: A $\alpha$ , B $\beta$  and  $\gamma$ . [14] The 3 fibrinogen chains are encoded by 3 separate, closely linked genes situated on the same chromosome and located in the sequence  $\gamma$ , A $\alpha$ , and B $\beta$ , with the last one in opposite transcriptional orientation to the first one.[15] Binsack et al [16] reported that in the human hepatoma cell line, HepG2, bezafibrate suppressed A $\alpha$ -, B $\beta$ - and  $\gamma$ - chain mRNA levels.[17] The genes encoding the 3 fibrinogen chains are negatively regulated by PPAR $\alpha$  and since fibrates act through these receptors, it helps to explain the benefits of fibrate therapy to target a reduction in fibrinogen levels. Maison et al<sup>18</sup> showed that fibrinogen concentration decreased after fibrate therapy, while it increased after statin treatment. Thus this study was conducted to investigate the controversial role of statins in modifying fibrinogen levels and to study the benefits of addition of fenofibrate to statin therapy in modifying levels of plasma fibrinogen.

## Research Design and Methods

The study was a controlled clinical trial. It was randomized, non blind and based on parallel group design. The protocol of the study received approval from the Institutional Review Board of the Sterling Hospital, Ahmedabad. Patients of ACS of either sex who had undergone Percutaneous Transluminal Coronary Angioplasty (PTCA) procedure irrespective of the presence of diabetes mellitus were included in the study after taking their consent. Patients with second or third degree AV (atrioventricular) block, renal or hepatic failure, recent cerebrovascular events, valve replacement surgery, or Balloon Mitral Valvuloplasty (BMV) and patients on lipid lowering therapy other than statins were excluded from the study. 102 consecutive patients meeting the eligibility criteria were randomized into four treatment groups, consisting of patients given atorvastatin, simvastatin, atorvastatin-fenofibrate combination and simvastatin-fenofibrate combination. Treatment was started after the PTCA and was continued for 3 months. Atorvastatin was given in a single dose of 20mg per day (if alone) and 10mg per day (in combination with fenofibrate), simvastatin was given in a single dose of 40mg per day (if alone) and 20mg per day (in combination with fenofibrate) and fenofibrate was given in a single dose of 200mg per day. The first blood samples were collected before the onset of the treatment and were analyzed for total cholesterol, triglycerides(TG), LDL, HDL, VLDL and plasma fibrinogen. The second blood samples were collected after 3 months of treatment for total cholesterol, triglycerides, HDL, LDL, VLDL, plasma fibrinogen and liver function tests that included SGPT, SGOT, total bilirubin and alkaline phosphatase. Total cholesterol, TG, HDL-C, SGPT, SGOT, total bilirubin and alkaline phosphatase were analyzed on automated VITROS 250 analyzer using enzymatic assay methods. LDL-C was analyzed by enzymatic method. VLDL-C was calculated using Friedewald's formula.

Plasma fibrinogen was assayed using Immunoprecipitation method using the RANDOX® kits. Many studies measure fibrinogen by the Clauss Method. However, data from the Framingham Heart Study [19] suggest that levels determined by the immunoprecipitation test have a stronger association with cardiovascular disease than those obtained by

Clauss method. The results were analyzed by applying Student's t test, ANOVA and linear regression to find out degree of correlation between parameters. The probability value of less than 5% ( $p<0.05$ ) was considered to be statistically significant.

## Results

We included in our study, 102 patients from the cardiology unit of Sterling hospital. All of them had angiographically documented CAD and had undergone PTCA. 25 patients each were enrolled in atorvastatin, atorvastatin-fenofibrate combination and simvastatin-fenofibrate combination treatment groups and 27 patients were enrolled in simvastatin treatment group. We had 4 dropouts in atorvastatin-fenofibrate group as well as in simvastatin monotherapy group and 3 dropouts in simvastatin-fenofibrate group, all due to non-technical reasons. So, the analysis is based on a total of 91 patients who completed the follow-up successfully. The baseline, demographic characteristics like age, sex, BMI, smoking/tobacco/alcohol habits, past history of diabetes, hypertension, family history etc. and other hemodynamic parameters like hemoglobin, urea, creatinine, etc were recorded (Table 1). These parameters were found to be identical in all the four treatment groups indicating a symmetric study design and population.

The effects of all the treatments on lipid parameters are given in Table 2. Atorvastatin and simvastatin monotherapy reduced serum LDL-C and interestingly increased serum HDL-C significantly ( $p<0.05$ ). Atorvastatin-fenofibrate combination produced a significant decrease in TG, VLDL ( $p<0.05$ ) and also increased HDL-C significantly as compared to atorvastatin alone ( $p<0.05$ ). Similar to simvastatin monotherapy, simvastatin-fenofibrate combination produced a significant increase in HDL and decrease in LDL levels ( $p<0.05$ ). It, however, did not reduce TG significantly. All the four treatments significantly reduced total cholesterol/HDL ratio and LDL/HDL ratio ( $p<0.05$ ). ANOVA test was applied to find if there was any difference in the effects between the four groups. The results however, indicated that the percentage change in lipoprotein levels obtained were not significantly different from each other between the four treatment groups (Figure 1).

**Table 1. Baseline Demographic and Haemodynamic characteristics of the Patients**

Characteristics	Atorvastatin (n=25)	Atorvastatin + Fenofibrate (n=25)	Simvastatin (n=27)	Simvastatin + Fenofibrate (n=25)
Age(years)	56.76 ± 9.4	56.44 ± 9.95	58.44 ± 11.4	58.35 ± 11.35
Females (%)	4%	12 %	7.4%	20%
Males (%)	96%	88%	92.6 %	80%
Body Mass Index(BMI)	25.79 ± 3.5	23.62 ± 1.95	25.3 ± 3.90	25.44 ± 4.25
Smokers	12%	20%	14.81 %	8%
Tobacco Chewers	12%	12%	7.4 %	4%
Diabetics	24%	36%	29.62%	48%
Hypertensives	28%	28%	51.85 %	40%
Haemoglobin (gm%)	12.61± 1.6	12.17± 1.75	13.16± 1.76	13.07± 1.65
Urea (mg/dl)	30.7± 18.9	26.81± 11.95	26.93± 15.46	26.91± 13.2
Creatinine (mg/dl)	1.23± 0.5	1.28± 1.1	1.10± 0.31	1.10± 0.3
Random bl. Sugar (mg/dl)	130.33± 39.25	146.73± 65.45	137.8 ± 57.45	137.48 ± 56.6
Systolic BP (mm Hg)	126.54 ± 16.05	129.52 ± 24.25	128.22 ± 22.47	128.84 ± 22.8
Diastolic BP (mm Hg)	81.29 ± 8.75	78.35 ± 9.00	80.55 ± 11.31	80.96 ± 11.55
Pulse	77.09 ± 15.4	80.55 ± 10.9	75.02 ± 15.46	75.29 ± 15.65
LVEF (%)	46.8 ± 12.35	50.94 ± 16.25	52.44 ± 12.79	52.51 ± 5.76
Single Vessel disease	68%	56%	55.55%	44%
Double vessel disease	28%	28%	29.62 %	36%
Triple vessel disease	4%	16%	14.81%	20%

Data expressed as mean ± SD; % of patients

**Table 2. Effect of treatments of serum lipoprotein levels**

Parameters	Time interval	Atorvastatin <sup>1</sup> (n=25)	Atorvastatin + Fenofibrate <sup>2</sup> (n=21)	Simvastatin <sup>3</sup> (n=23)	Simvastatin + fenofibrate <sup>4</sup> (n=22)
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Total cholesterol mg/dl	baseline	156.6 ± 34.15	150.9± 46.39	150.44 ± 31.1	148.9 ± 31.98
	3 months	149.68 ± 42.8	155.76± 25.42	146.42±53.65	139.74 ± 30.67
TG mg/dl	baseline	144.85±76.65	155.1±112.12	138.5 ± 78.55	136.43 ± 78.97
	3 months	133.86±61.85	108.5±47.63*	123.5±100.7	110.12±47.5
HDL-C mg/dl	baseline	37.69 ± 9.1	35.34 ± 9.25	34.91 ± 8.57	34.88 ± 8.67
	3 months	41.55 ± 9.2*	46.61±8.38*	43.74 ± 12.9*	43.74 ± 13.27*
LDL-C mg/dl	baseline	101.44 ± 33.5	91.11 ± 33.06	93.39± 23.33	92.10 ± 24.10
	3 months	82.49 ±34.5*	86.74 ± 18.64	76.48 ± 26.3*	76.48 ± 26.35*
VLDL-C mg/dl	baseline	28.96 ±15.2	31.0 ± 22.39	27.68 ± 15.71	27.78 ± 15.85
	3 months	26.87 ± 12.35	21.71±9.66*	24.27 ± 19.97	24.17 ± 20.02
LDL- C/HDL-C	baseline	2.87 ± 1.35	2.66 ± 0.82	2.89 ± 1.05	3.13 ± 1.92
	3 months	2.06 ±0.95*	1.91 ± 0.50*	1.92 ± 0.81*	1.92 ± 0.79*
total cholesterol/ HDL-C	baseline	4.33 ± 1.25	4.41 ± 1.42	4.42 ± 1.29	4.37 ± 1.31
	3 months	3.71 ± 1.2 *	3.42 ± 0.69	3.66 ± 1.77*	3.66 ± 1.78*

Baseline: before starting of treatment

3 months: after 3 months of treatment

\* Significant change from baseline values; student's t test, p<0.05;

df<sup>1</sup>=24, df<sup>2</sup>=20, df<sup>3</sup>=22, df<sup>4</sup>=21 (degree of freedom)

**Table 3. Effect of treatments on plasma fibrinogen levels**

Plasma Fibrinogen Gm/Lt	Atorvastatin <sup>1</sup> (n=25)	Atorvastatin + Fenofibrate <sup>2</sup> (n=21)	Simvastatin <sup>3</sup> (n=23)	Simvastatin + fenofibrate <sup>4</sup> (n=22)
Initial Levels	4.44 ± 0.26	4.29 ± 0.4	4.25 ± 0.25	4.21 ± 0.27
After 3 months	3.47 ± 0.18 *	3.10 ± 0.28*	3.12 ± 0.14*	3.12 ± 0.15 *

\*significant change from baseline values; Paired Student's t test, p<0.05

df<sup>1</sup> = 24, df<sup>2</sup> = 20, df<sup>3</sup> = 22, df<sup>4</sup> = 21

**Table 4. Correlation coefficient between fibrinogen and other study parameters**

Parameters	Correlation coefficient (r)
Body Mass Index (BMI)	-0.02
Haemoglobin	-0.21
Urea	0.18
Creatinine	-0.21
Blood Glucose	-0.14
Cholesterol	0.05
Triglycerides	0.01
HDL-C	-0.10
LDL-C	0.07
VLDL-C	-0.02
LDL-C/HDL-C	0.10
CHO/HDL-C	0.10

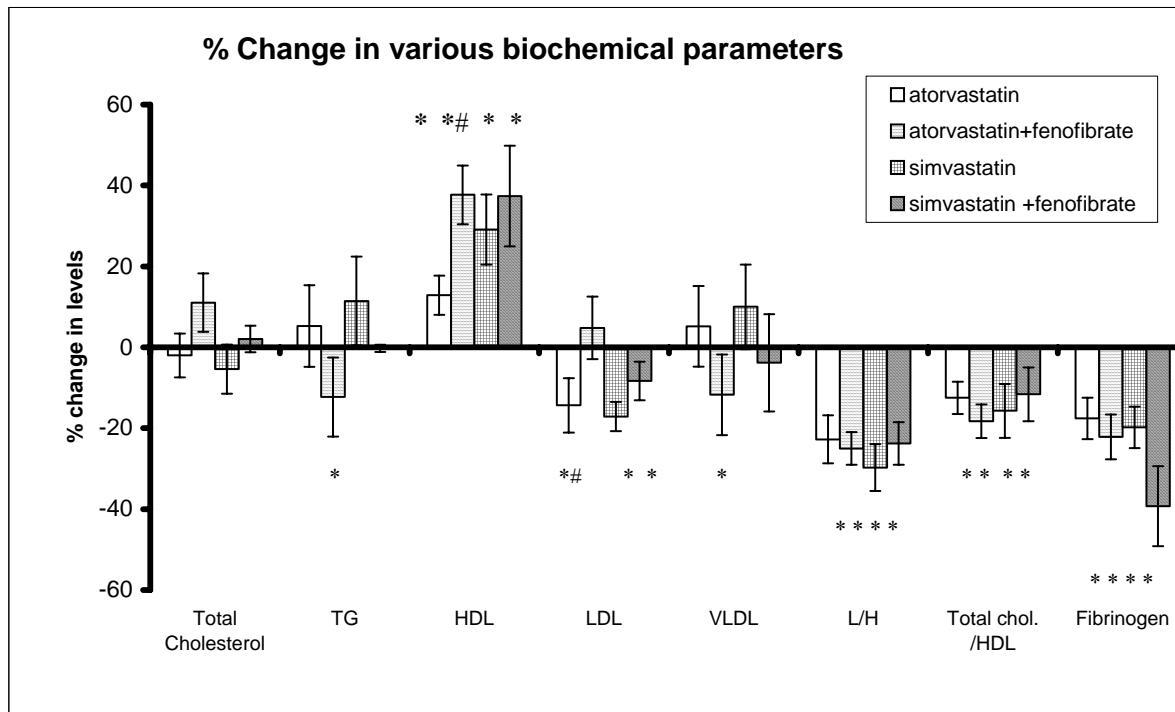
**Table 5. Effect of treatments of Liver Function Parameters at the end of three months**

Parameters	Atorvastatin <sup>1</sup> (n=25)	Atorvastatin + Fenofibrate <sup>2</sup> (n=21)	Simvastatin <sup>3</sup> (n=23)	Simvastatin + fenofibrate <sup>4</sup> (n=22)
		Mean ± SD	Mean ± SD	Mean ± SD
Total bilirubin mg/dl	0.7 ± 0.25	0.67 ± 0.18	0.71 ± 0.28	0.71 ± 0.28
SGPT U/Lt	28.16 ± 10.9	29.62 ± 7.19	31.67 ± 7.99	31.48 ± 1.72
SGOT U/Lt	24.46 ± 7.1	29.14 ± 6.59	27.62 ± 7.28	27.55 ± 1.56
Alk.Phosph-atase U/Lt	94.04 ± 23.05	69.00 ± 16.48	74.51 ± 26.25	73.80 ± 5.42

Data expressed as Mean ± SD

\*Significant change from baseline values; student's t test, p<0.05;

df<sup>1</sup>=24, df<sup>2</sup>=20, df<sup>3</sup>=22, df<sup>4</sup>=21 (degree of freedom)



\*Significant change from baseline values, Student's t test,  $p<0.05$ .

# Significant change as compared to the respective statin monotherapy or combination,

Unpaired Student's t test,  $p<0.05$

ANOVA test applied on % change in levels,  $P>0.05$ , Changes are not significantly different from each other between the groups

Figure 1: % change in lipid levels between both the treatment group.

The effect of the treatments on plasma fibrinogen are given in Table 3. Atorvastatin as well as simvastatin was found to decrease plasma fibrinogen significantly ( $p<0.01$ ). Combination of statins with fenofibrate also showed a significant decrease in plasma fibrinogen ( $p<0.01$ ). However, the treatment groups were not significantly different from each other when analyzed by ANOVA test. Thus, contrary to reports, statins in our study decreased plasma fibrinogen significantly while addition of fenofibrate enhanced this effect, however significantly.

No correlation was found between fibrinogen levels and various demographical, haemodynamic and biochemical parameters (Table 4). Thus, plasma fibrinogen was found to be an independent risk factor for CAD and was lowered significantly by atorvastatin and simvastatin alone and in combination with fenofibrate.

No significant elevations in the serum bilirubin concentration, transaminases (SGPT, SGOT) and

alkaline phosphatase levels were observed in any treatment group (Table 5). It thus appeared that all the treatments were safely tolerated in the given doses without any adverse hepatotoxic effects.

## Discussion

Statins alone as well as in combination with fenofibrate were found to produce beneficial effects in CAD patients after PTCA. Statins are potent inhibitors of HMG Co-A reductase, which decreases LDL-C by upregulating LDL receptor activity in the liver and reducing the secretion of apolipoprotein B containing lipoprotein. The latter is believed to be responsible for the TG lowering effect of atorvastatin and is profound at higher doses.[20] Various reports have indicated that atorvastatin lowers LDL-C and increases HDL.[21,22,23] The effects of atorvastatin in our study comply with such reports. On the other

hand, simvastatin is reported to produce a decrease in LDL-C from 26% to 50% in various doses.[24, 25] Also, reports from Hunninghake et al [26] showed that simvastatin consistently produces a larger increase in HDL-C as compared to atorvastatin. Results from our study comply with the above findings as simvastatin in our study too produced a significant decrease in LDL-C and increase in HDL-C as compared to atorvastatin.

Fibrates activate PPAR- $\alpha$  which ultimately leads to their HDL raising and TG lowering effects.[27,28] Certain previous studies have suggested increased benefits of a combination of fenofibrate with statins. [29,30] Reports are available that show benefits of combining fenofibrate and simvastatin. [30, 31] A study of the literature also shows that a greater change in HDL and TG levels is obtained with fenofibrate - atorvastatin combination as compared to monotherapy [32] with either drug. Our results comply with reported findings. We observed that combining fenofibrate with statins offered a greater benefit in reducing TG and VLDL levels as well as increasing HDL levels. Thus, the combination therapy proved both safe and beneficial in our patients with ACS.

In prospective studies, plasma fibrinogen has been found to be an independent predictor of myocardial infarction in both genders. It provides information on CAD risk over and beyond that supplied by established risk factors. [33] The Framingham study has shown that for both sexes, the risk of cardiovascular diseases was correlated positively to antecedent fibrinogen values higher than 1.3 to 7.0 gm/L.[34] We found that fibrinogen in our study did not correlate with any of our study parameters including demographic characters and lipid levels. Thus, fibrinogen does appear to be an independent risk factor for CAD.

Reports from large-scale trials consistently show statins to have a neutral effect on fibrinogen. [35,36] Various studies on plasma fibrinogen indicate that fenofibrate lowers fibrinogen levels but the effects of atorvastatin and simvastatin have been variable and controversial, particularly that of atorvastatin. Wierzbicki et al [37] showed that atorvastatin increases plasma fibrinogen by 22%. Song et al. [38] also found a significant increase in fibrinogen with atorvastatin treatment where as simvastatin was reported to have a neutral effect. Various reports

indicated that fenofibrate decreased plasma fibrinogen significantly whereas atorvastatin produced an increase in fibrinogen levels.[39, 40,41] Otto et al [42] showed that plasma fibrinogen and other hemorheologic parameters were unchanged during atorvastatin treatment in comparison to simvastatin treatment. Athyros et al. [43] reported that plasma fibrinogen was unaffected by atorvastatin and was significantly reduced by fenofibrate and combination of both. Ceska et al [44] showed that fenofibrate is a potent hypolipidemic drug with only rare side effects and reduces fibrinogen significantly. In our study, however, contrary to all above reports for statins, we observed that atorvastatin as well as simvastatin produced a significant decrease in plasma fibrinogen levels. However, our results comply with some recent findings by Kadikoylu and co- workers that show a decrease in plasma fibrinogen by atorvastatin and simvastatin. [45] Another study by Leibovitz et al. [47] proved that atorvastatin reduces fibrinogen levels in patients with severe hypercholesterolemia. fibrinogen levels dropped by almost 18% in this study. Tekin et al [48] has also shown a reduction in plasma fibrinogen levels by atorvastatin in hyperlipidemic patients with angiographically proved CAD. These findings with atorvastatin are consistent with such recent data. The combination of statins with fenofibrate also produced a further decrease in fibrinogen, though not significant. These results are in agreement with the reported claims of fenofibrate to decrease plasma fibrinogen.

Previous studies have indicated an increased risk of myopathy with statin-fibrate combination. [29,46] However, no cessation of therapy was required in any patient due to such complications in our study. Liver function tests of all patients were also normal at the end of 3 months of treatments indicating no adverse hepatotoxic effects. These findings suggest that it may be safe to use fenofibrate and statins in combination contrary to reported contra- indications for the combined use of these two classes of drugs. However, we have not looked into the long-term toxicity associated with the use of these drugs in combination. Moreover, the strategy appears to be distinctly beneficial in lowering the risk factor plasma fibrinogen. Thus, the therapy offers a new method to treat patients with acute coronary syndromes.

## Conclusions

Addition of fenofibrate to statins seems to be beneficial in patients with ACS. Statins, contrary to various reports, were found to decrease plasma fibrinogen significantly. Also, in combination with fenofibrate there was enhanced reduction of the novel risk factor, fibrinogen.

## References

- [1] Murray JL, Lopez AL. Alternative projections of mortality and disability by cause 1990-2020. Global Burden of Disease Study. *Lancet* 1997; 349:1498-1504.
- [2] Tsimihodimos V, Kostoula A, Kakafika A, Bairaktari E, Tselepis AD, Mikhailidis DP, Elisaf M. Effect of fenofibrate on serum inflammatory markers in patients with high triglyceride values. *J Cardiovasc Pharmacol Ther* 2004; 9:27-33.
- [3] Kockx M, Gervois PP, Poulain P, Derudas B, Peters JM, Gonzalez FJ, Princen HM, Kooistra T, Staels B. Fibrates suppress fibrinogen gene expression in rodents via activation of the peroxisome proliferators-activated receptor-alpha. *Blood* 1999; 93: 2991-8.
- [4] Green F, Humphries S. Control of plasma fibrinogen levels. *Baillieres Clin Haematol* 1989;2: 945-59.
- [5] Woods A, Brull DJ, Humphries SE, Montgomery HE. Genetics of inflammation and risk of coronary artery disease: the central role of interleukin-6. *Eur Heart J* 2002; 21: 1574-83.
- [6] Shepherd, J., Cobbe, S. M., Ford, I. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N Engl J Med* 1995; 333: 1301-7.
- [7] Sacks, F. M., Pfeffer, M. A., Moye, L. A. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. *N Engl J Med* 1996 335:1001-9.
- [8] The Scandinavian Simvastatin Survival Group. Randomized trial of cholesterol lowering in 4444 patients with coronary heart disease: The Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994; 344: 1383-9.
- [9] Athyros, V. G., Papageorgiou, A. A., Hatzikonstandinou, H. A., Athyrou, V. V., Kontopoulos, A. G. Effect of atorvastatin versus simvastatin on lipid profile and plasma fibrinogen in patients with hypercholesterolemia. *Clin Drug Inves* 1998; 16: 219-27.
- [10] Rosenson RS, Tangney CC, Casey LC. Inhibition of proinflammatory cytokine production by pravastatin. *Lancet* 1999; 353: 983-4.
- [11] Rosenson RS, Tangney CC. Anti-atherothrombotic properties of statins: implications for cardiovascular event reduction. *JAMA* 1998; 279:1643-50.
- [12] Handley DA, Hughes TE. Pharmacological approaches and strategies for therapeutic modulation of fibrinogen. *Thromb Res* 1997; 87:1-5.
- [13] Ernst E, Resch KL. Therapeutic interventions to lower plasma fibrinogen concentration. *Eur Heart J* 1995; 16:47-51.
- [14] Fuller GM. Fibrinogen: a multifunctional acute phase protein, in Mackiewicz A, Kushner I, Baumann H (eds): Acute phase proteins: Molecular Biology, biochemistry and clinical applications, New York, NY, Doubleday, 1993,p 169.
- [15] Kant JA, Fornace AJ, Saxe D, Simon MI, McBride OW, Crabtree GR. Evolution and organization of the fibrinogen locus on chromosome 4: Gene duplication accompanied by transposition and inversion. *Proc Natl Acad Sci USA* 1985; 82: 2344-50
- [16] Binsack R, Stegmeier K, Dorge L, Volkl A. Bezafibrate down-regulates fibrinogen biosynthesis in human hepatoma HepG2 cells. *Eur J Clin Invest* 1998; 28:151-5.
- [17] Roy SN, Mukhopadhyay G, Redman CM. Regulation of fibrinogen assembly. Transfection of Hep G2 cells with B beta cDNA specifically enhances synthesis of the 3 component chains of fibrinogen. *J Biol Chem* 1990; 265:6389-93.
- [18] Maison, P., Mennon, L., Sapinho, D., Balkau, B., Sigalas, J., Chesnier, M. C., Eschwege, E. D.E.S.I.R. Study group. A pharmacoepidemiological assessment of the effect of statins and fibrates on fibrinogen concentration. *Atheroscler* 2002; 160:155-60
- [19] Stec JJ, Silbershatz H, Tofler GH, Matheney TH, Sutherland P, Lipinska I, Massaro JM, Wilson PF, Muller JE, D'Agostino RB Sr. Association of fibrinogen with cardiovascular risk factors and cardiovascular disease in the Framingham Offspring Population. *Circ* 2000; 102: 1634-8.
- [20] Burnett JR, Wilcox LT, Telford DE, Kleinstiver SJ, Barrett PHR, Newton RS, Huff MW. Inhibition of HMG Co A reductase by atorvastatin decreases both VLDL and LDL, apoB production in miniature pigs. *Arterioscler Thromb Vasc Biol* 1997;17:2489-2500.
- [21] Haw N, Fowler G, Patel H, Eminton Z, Maton S. An assessment of the efficacy of atorvastatin in achieving LDL cholesterol target levels in patients with coronary heart disease: a general practice study. *Int J Clin Pract* 1999;53:422-6.
- [22] Marais AD, Firth JC, Bateman M, Jones J, Mountney J. Atorvastatin is a powerful and safe agent for lowering plasma cholesterol concentration in heterozygous familial hypercholesterolemia. *Atheroscler* 1994;109:316-20.
- [23] Frost R, Otto C, Geiss C, Schwandt P, Parhofer KG. Effects of atorvastatin versus fenofibrate on lipoprotein profiles, low density lipoprotein subfraction distribution, and hemorheologic parameters in type 2 diabetes mellitus with mixed hyperlipoproteinemia. *Am J Cardiol* 2001;87:44-8.

- [24] Jones, P., Kafonek, S., Laurova, I. Comparative dose efficacy study of atorvastatin versus simvastatin, pravastatin, lovastatin and fluvastatin in patients with hypercholesterolemia. (The CURVES Study). *Am J Cardiol* 1998; 81: 582-7.
- [25] Crouse JR III, Frolich J, Ose L, Mercuri M, Tobert JA. Effects of high doses of simvastatin and atorvastatin on high-density lipoprotein cholesterol and apolipoprotein A-1. *Am J Cardiol* 1999;83:1476-7.
- [26] Hunninghake DB, Ballantyne CM, MacCubbin DL, Shah AK, Gumbiner B, Mitchel YB. Comparative effects of simvastatin and atorvastatin in hypercholesterolemic patients with characteristics of metabolic syndrome. *Clin Ther* 2003;25:1670-86.
- [27] Staels B, Dallongvile J, Auwerx J, Schoonjans K, Leitersdorf E, Fruchart JC. Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circ* 1998;98:2088-93.
- [28] Watts GF, Dimmit SB. Fibrates, dyslipoproteinemia and cardiovascular disease. *Curr Opin Lipid* 1999;10:561-74.
- [29] Athyros VG, Papageorgiou AA, Hatzikonstantinou HA, Didangelos TP, Carina MV, Kranitsas DF, Kontopoulos AG. Safety and efficacy of long term statin-fibrate combination in patients with refractory familial combined hyperlipidemia. *Amer J Cardiol* 1997; 80: 608-13.
- [30] Ruth LBE and Ruth McPherson. Long term efficacy and safety of fenofibrate and statin in treatment of combined hyperlipidemia. *Amer J Cardiol* 1998; 81: 60B-65B.
- [31] Vega GL, Ma PT, Cater NB, Fillipchuk N, Megura S, Garcia AB, Grundy SM. Effects of adding fenofibrate (200mg/day) in patients with combined hyperlipidemia and metabolic syndrome. *Amer J Cardiol* 2003; 91: 956-60.
- [32] Keating GM, Ormod D. Micronised fenofibrate: an updated review of its clinical efficacy in the management of dyslipidaemia. *Drugs* 2002; 62:1909-44.
- [33] Heinrich, J., Assmann, G. Fibrinogen and cardiovascular risk. *J Cardiovasc Risk* 1995;2: 197-205.
- [34] Kannel, W. B., Neaton, J. D., Wentworth, D., Thomas, H. E., Stamler, J., Hulley, S. B. For the MRFIT Research group. Overall and coronary heart disease mortality rates in relation to major risk factors in 325,348 men screened for the MRFIT trial. Multiple Risk Factor Intervention Trial. *Am Heart J* 1987; 112:825-36.
- [35] Rosenson RS, Koenig W. Utility of inflammatory markers in the management of Coronary artery disease. *Amer J Cardiol* 2003; 92:10i-18i.
- [36] Lowe G, Rumley A, Norrie J, Ford I, Shepherd J, Cobbe S, Macfarlane P, Packard C, on behalf of the West of Scotland Coronary Prevention Study Group. Blood Rheology, cardiovascular risk factors and cardiovascular disease: the west of Scotland Coronary Prevention Study. *Thromb Haemost* 2000; 84: 553-8.
- [37] Wierzbicki, A. S., Lumb, P. J., Semra, Y. K. Effect of atorvastatin on plasma fibrinogen. *Lancet* 1998; 351:569-70.
- [38] Song, J. C., White, C. M. Do HMG CoA reductase inhibitors affect fibrinogen? *Ann Pharmacother* 2001; 35:236-41.
- [39] Bairaktari, E. T., Tzallas, C. S., Tsimihodimos, V. K., Liberopoulos, E. N., Miltiadous, G. A., Elisaf, M. S. Comparison of the efficacy of atorvastatin and micronized fenofibrate in the treatment of mixed hyperlipidemia. *J Cardiovasc Risk* 1999; 6:113-6.
- [40] Frost, R., Otto, C., Geiss, C., Schwandt, P., Parhofer, K. G. Effects of atorvastatin versus fenofibrate on lipoprotein profiles, low density lipoprotein subfraction distribution, and hemorheologic parameters in type 2 diabetes mellitus with mixed hyperlipoproteinemia. *Am J Cardiol* 2001; 87:44-8.
- [41] Serna, G., Cadarso, C. Fenofibrate decreases plasma fibrinogen, improves lipid profile, and reduces uricemia. *Clin Pharmacol Ther* 1999; 66:166-72.
- [42] Otto, C., Geiss, H. C., Donner, M. G., Parhofer, K. G., Schwandt, P. Influence of atorvastatin versus simvastatin on fibrinogen and other hemorheological parameters in patients with severe hypercholesterolemia treated with regular low density lipoprotein immunoadsorption apheresis. *Ther Apher* 2000; 4:244-8.
- [43] Athyros, V. G., Papageorgiou, A. A., Athyrou, V. V., Demitriadis, D. S., Kontopoulos, A. G. Atorvastatin and micronized fenofibrate alone and in combination in type 2 diabetes with combined hyperlipidemia. *Diab Care* 2002; 25:1198-1202.
- [44] Ceska R, Sobra J, Kvasnicka J, Prochazkova R, Kvasilova M, Haas T. The effect of micronized fenofibrate on lipid parameters and fibrinogen in heterozygous familial hypercholesterolemia and familial combined hyperlipidemia. *Cas Lek Cesk* 1996;135: 413-6.
- [45] Kadikoylu, G., Yukselen, V., Yavasoglu, I., Bolaman, Z. Hemostatic effects of atorvastatin versus simvastatin. *Ann Pharmacother* 2003; 37:478-84.
- [46] Tikkanen MJ. Statins within-group comparisons, statin escape and combination therapy. *Curr Opin Lipidol* 1996; 7: 385-8.
- [47] Leibovitz E, Hazanov N, Frieman A, Elly I, Gavish D. Atorvastatin reduces fibrinogen levels in patients with severe hypercholesterolemia: additional evidence to support the anti-inflammatory effects of statins. *Isr Med Assoc J* 2004;6:456-9.
- [48] Tekin A, Tekin G, Guzelsoy D, Kaya A, Gurel CV, Yigit Z, Ulutin T. Effects of atorvastatin (10 mg) on hemostatic and inflammatory parameters in hyperlipidemic patients with angiographically proven coronary artery disease. *Am J Cardiol*. 2004;94:206-9.





## ARTICLE

# Effects of Sildenafil in Cardiovascular Disease

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## Abstract

Recent studies indicate that sildenafil is cardioprotective by preconditioning of the myocardial and endothelial cells. In the present review, we examine the available evidence regarding this beneficial effect. Experimental studies indicate that sildenafil can provide beneficial effects to the myocardium and endothelium by enhancing preconditioning. There are a few clinical studies among healthy subjects and a few observational studies indicating that sildenafil is useful in patients with poor endothelial function and in those with myocardial ischaemia. We found among 6 volunteer patients with nonST-elevation myocardial infarction that treatment with sildenafil was protective in limiting infarct size. We also observed that sildenafil can decrease blood pressure among hypertensives and provide benefits against heart failure in patients with acute left ventricular failure.

Experimental and clinical experiences support the usefulness of sildenafil in patients with acute myocardial infarction, heart failure and hypertension. Randomized, controlled trials and long-term follow-up are necessary to confirm these observations.

**Keywords:** sildenafil,cardioprotective,hypertension,heart failure,nitric oxide.

## Introduction

There is consistent evidence that sildenafil can protect against endothelial damage and myocardial injury by enhancing preconditioning [1, 2]. One study showed [2], that opening of mitochondrial ATP sensitive K channels by ischemic preconditioning or Diazoxide protected the endothelium by reducing the bursts of free radicals that occurred at reperfusion. Mitochondria is rich in the coenzyme Q10 and carnitine, which are cytoprotective antioxidants and bioenergetic agents [3-5]. Both these agents are known to maintain ATP, which poses the possibility that endogenous levels of the agents or their

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supplementation may be protective against free radical-induced damage to ATP Sensitive K channels. It is also possible that these agents might enhance the capability of the myocardial and endothelial cells in preconditioning which is known to protect the cells against injury responsible for arrhythmias, and can modulate remodeling of the myocardium and endothelium against heart failure and atherogenesis.[4-6]. It has been shown that this widely-used drug for treating erectile dysfunction reduces the apoptosis under heart attack-like conditions in a laboratory model. These findings may help researchers to develop a new treatment for patients with heart failure and acute myocardial infarction (AMI), where the loss of cells is primarily due to cell suicide or ischemic damage respectively [5, 6].

## Clinical Observations

In our experience, 3 patients, aged 45-60 years, (1 male and 2 females), presenting with congestive heart failure due to hypertension (systolic >200mmHg and diastolic >110mmHg) and ischemic heart disease, were given furosemide 40mg intravenously daily plus sildenafil 50mg twice daily, plus perindopril 4-8mg daily, there was a marked improvement in New York Heart Association(NYHA) class heart failure and quality of life including improvement in fatigue and dyspnea within 48 hours of treatment. All 3 patients were discharged by the end of one week. All these patients had radiological, electrocardiographic and echocardiographic manifestations of heart failure, including lower ejection fraction between 40-50%. There was a significant reduction in systolic and diastolic blood pressures in all the patients (>150/95mmHg).

In another clinical observation, among 6 patients with acute myocardial infarction(AMI),we noted that sildenafil (25mg 2-3 times, daily) can decrease anginal episodes, cause less increase in LDH and reduce electocardiographic QRS score( infarct size) when compared to another 6 age, sex and body mass index matched subjects not given this agent. There was a 20-25% increase in heart rate and decrease in blood pressures initially among patients receiving sildenafil compared to the baseline heart rate, which

settled to baseline rates after about 5 days treatment. The patients in both groups also did not receive streptokinase because some of them presented beyond 20-24 hours of onset of chest pain and some had nonST-segment elevation AMI. Their age varied between 45-60 years with 5 males and 1 female in each group. One patient with inferior wall infarction had hypotension, (80/50mmHg) where the dose of sildenafil was decreased to 25mg/day from 25mg thrice daily. There were no adverse effects of sildenafil in both of our observations and all patients gave written informed consent for the administration of sildenafil.

## Existing Clinical Evidence

In one study among healthy subjects [7], ischemia/reperfusion on endothelial and neutrophil function was studied, in which the forearm was made ischemic by inflating a blood pressure cuff to 200mmHg for 20 minutes. The dilator response to the endothelial dependent dilator, acetylenecholine and the nonendothelial dependent dilator nitroglycerine was determined before and after ischemia/reperfusion. Neutrophil function was studied by determining the upregulation of the CD11b in the circulating blood. Ischemic preconditioning was developed by 3 five minute periods of ischemia/reperfusion prior to the 20 minute ischemic periods. There was a marked decline in radial artery dilation to acetylenecholine but not to nitroglycerine and an upregulation of CD11b, due to ischemia/reperfusion in the control subjects. Ischemic preconditioning blunted these responses to ischemia/reperfusion. This study suggests possible pathways of pharmacological preconditioning of the vasculature causing better reflow in patients with endothelial injury. In another more recent study [8], the role of ATP sensitive K channels was studied in mediating the influence of ischemic preconditioning among healthy volunteers after ischemia/reperfusion of the forearm. Intraarterial administration of acetylenecholine was used to determine vasodilator response, indicating endothelial function, before and at 15 minutes, after 20 minutes of forearm ischemia/reperfusion under the influence of various drugs. Ischemic preconditioning protected against induced endothelial dysfunction by

ischemia/reperfusion and this protection was blunted by cotherapy with glibenclamide. However, pretreatment with diazoxide simulated the effects of preconditioning to protect against endothelial dysfunction, which was blocked by glibenclamide. Interestingly, in the absence of ischemia/reperfusion, diazoxide had no influence on vasodilator responses to acetylcholine. It is possible that ATP sensitive K channels have an important role in ischemia/reperfusion-induced endothelial damage in humans. It is also likely that modulation of channel pathways using certain agents by activation of K channels may have a therapeutic use in patients with AMI. Tomai et al [9], in one early study, reported that repeated episodes of angioplasty induced ischemia blocked by glibenclamide, had no beneficial influence on cardiomyocytes that was to be provided by preconditioning. These clinical studies and our observations in AMI indicate that activation of myocardial and and/or endothelial localized ATP sensitive K channels are useful in patients with coronary artery disease, which might decrease injury to endothelial cells as well as to cardiomyocytes. In a most recent observation, Gori et al [10] studied the effects of treatment with sildenafil (50mg/day) among 10 healthy male volunteers, 2 hours prior to 15 minutes of cuff-induced radial artery ischemia, followed by 15 minutes of reperfusion. In a double blind, randomized, crossover study, flow mediated vasodilation was determined prior to drug or placebo treatment as an indicator of endothelial function. Glibenclamide (5mg) was administered one hour prior to sildenafil administration in a randomized, double blind fashion, in a separate group of 7 healthy volunteers. There was a significant reduction in the endothelial function in placebo-treated controls and this decline was prevented by prior treatment with sildenafil. However, the beneficial effect of sildenafil was blunted by glibenclamide at a dose, which had no influence on endothelial function in the absence of sildenafil. This study has provided proof for the first time that sildenafil provides protection to the endothelium against ischemia/reperfusion in humans and this effect involves activation of vascular ATP sensitive K channels. One earlier study [11] also showed that treatment with sildenafil, acutely in patients with chronic heart failure enhances endothelium-dependent, flow mediated vasodilation

in the brachial artery, one hour after sildenafil therapy compared to control subjects. In another study [12], 100mg/day of sildenafil was administered causing dilation of epicardial coronary arteries, and improvement in endothelial function in the brachial artery and it also inhibited platelet function in patients with coronary artery disease. There was a significant reduction in exercise-induced ischemia upon treatment with sildenafil, which showed moderate effects when compared to placebo and isosorbide dinitrate. It is clear that sildenafil was effective, both in healthy volunteers as well as in patients with chronic heart failure and coronary artery disease [11-13]. Our observations do not provide proof that sildenafil should be administered in the treatment of AMI. However, in view of the above studies, it indicates a need for randomized, controlled trials to determine its exact role in the prevention of complications in these patients, especially in those who have myocardial ischemia in the presence of coronary atherosclerosis and left ventricular dysfunction. There are no serious adverse effects of sildenafil, except hypotension, especially in those patients receiving nitrates and other nitric oxide donors [14, 15].

In one study [16] data were pooled regarding myocardial infarction (MI) and cardiovascular death from more than 120 clinical trials of sildenafil citrate (Viagra) conducted from 1993 to 2001. During placebo-controlled trials, the rate of MI or cardiovascular death was 0.91 (95% CI: 0.52-1.48) per 100 person-years (PY) of follow-up among sildenafil-treated patients compared with 0.84 (95% CI: 0.39-1.60) per 100 PY of follow-up among placebo-treated patients. The relative risk of MI or cardiovascular death was 1.08 (95% CI: 0.45-2.77) for sildenafil compared with placebo ( $p = 0.88$ ). During open-label studies, the rate of MI or cardiovascular death was 0.56 (95% CI: 0.44-0.72) per 100 PY of follow-up. This analysis showed that the rates of MI and cardiovascular death were low and comparable between men treated with sildenafil and those treated with placebo. The use of sildenafil was not associated with an increase in the risk of MI or cardiovascular death.

## Experimental Evidence

Several studies [17-21] have examined the cardioprotective effects of sildenafil on myocardial infarct size and arrhythmias. All the studies reported a beneficial influence except one [17] showing that sildenafil-nitric oxide donor combination promotes ventricular tachyarrhythmias in a swine right ventricle. In one study [18], both acute treatment with sildenafil given 30 minutes prior to ischemia, and chronic administration, given 24 hours prior to the index ischemic period produced a marked preconditioning like effect in intact rabbits. After administration of the drug or the vehicle, rabbits were subjected to 30 minutes of ischemia and 3 hours of reperfusion. The triphenyletetraz -olium histochemical staining technique was used to determine the infarct size and showed that delayed treatment with sildenafil decreased the infarct size by 41% and acute treatment by 68%, respectively. Treatment with 5-HD, a mitochondrial selective, ATP sensitive K channel antagonist, 10 minutes prior to the index ischemic period, blocked these beneficial influences. Oral administration of sildenafil was associated with both acute and delayed cardiac protection.

Further studies [19, 20] showed that reduction of infarct size by sildenafil in acute administration was due to PKC and that an upregulation of both endothelial nitric oxide synthetase (eNOS) and iNOS occurred in a delayed preconditioning model in mouse hearts.

After 24 hours of sildenafil administration, treatment with a selective iNOS inhibitor, 1400W, antagonized the sildenafil-induced cardioprotection, if given 30 minutes prior to index ischemia. It is clear that PKC, nitric oxide and mitochondrial ATP sensitive K channels are involved in providing cardioprotective effects to reduce infarct size in experimental models.

Increased availability of coenzyme Q10 and carnitine may repair or strengthen these mechanisms, resulting in better protective effects.

Nagy et al [20] also showed that sildenafil decreased the severity of ventricular rhythm disturbances in experiments with dogs, 24 hours after oral treatment with this agent. However, when sildenafil was given in combination with nitric oxide

donor nitroprusside or nitroglycerine, it became arrhythmogenic in nonischemic, buffer-perfused, isolated right ventricular walls of swine hearts. Myocardial tissue deficiency of coenzyme Q10 and carnitine, which were not available in this experiment, may be the cause of these adverse effects. We also observed a 20-25% increase in baseline heart rate among patients with AMI receiving sildenafil. Other factors responsible for this discrepancy, may be due to protocol differences, a drug interaction or due to species differences between dogs and pigs.

## Mechanisms

Recently, it has been reported [2, 6] that treatment with sildenafil citrate at clinically relevant concentrations produced therapeutic levels of nitric oxide (NO) in the heart cells by increasing protein levels of two enzymes responsible for the synthesis of NO. Employing a cellular model where heart attack-like conditions are simulated in a Petri dish, it has been demonstrated that NO produced from sildenafil inhibits cell death by stabilizing mitochondria, thereby increasing the level of the anti-death protein, Bcl-2, and inhibiting caspase 3, the protein considered to be the ultimate weapon in cell suicide. This research has established a strong basis for the design of future studies targeted toward investigating the clinical effects of sildenafil on survival of the heart muscle following a major heart attack. In addition, these findings suggest that this drug may slow or possibly reverse the progressive loss of heart cells during chronic heart failure in patients with acute or chronic coronary artery disease. Researchers used heart cells prepared from genetically engineered mice that lack nitric oxide synthesizing enzymes. The model was particularly useful in studying the protective effect of sildenafil in heart muscle cells independent of any vascular effects or other types of cells. Sildenafil not only protected against necrosis, accidental cell death occurring due to oxygen deprivation, but also against apoptosis, cell death following DNA fragmentation. Furthermore, the researchers found the presence of phosphodiesterase-5 (PDE-5), an enzyme responsible for the destruction of the cGMP molecule, in the heart cells. The cGMP is an intracellular messenger molecule that plays an

important role in the dilation of arteries in the body. Sildenafil is able to preserve cGMP, and therefore dilation of the arteries by inhibiting PDE-5.

## Conclusion

These observations have far-reaching implications for the treatment of patients with heart failure where loss of cells is primarily due to apoptosis, and in patients with AMI and angina pectoris where the cell loss may be due to ischemic injury. In several other studies [6-21] and due to our observations, it is proposed that sildenafil has a powerful, protective effect in the heart during experimental and clinical heart attack in animal models, and in humans, respectively, indicating that it could be protective against AMI and hypertensive heart failure and also possibly in patients with hypertension for decreasing blood pressure. Randomized, controlled, clinical trials are necessary to demonstrate the role of sildenafil in cardiovascular disease.

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## References

- [1] Gross GJ. Sildenafil and endothelial dysfunction in humans (Editorial). *Circulation* 2005 (in press).
- [2] Beresewicz A, Maczewski M, Duda M. Effect of classical preconditioning and diazoxide on endothelial function and O<sub>2</sub> and NO generation in the post-ischemic guinea-pig heart. *Cardiovasc Res* 2004;63:118-129.
- [3] Chiang CA, Pella D, Singh RB. Coenzyme Q10 and adverse effects of statins. *J Nutr Environ Med* 2004;14:17-28.
- [4] Singh RB, Neki NS, Kartikey K, Niaz MA, Thakur AS. Effect of coenzyme Q10 on risk of atherosclerosis in patients with recent myocardial infarction. *Mol Cell Biochem* 2003;246:75-82.
- [5] Singh RB, Niaz MA, Agarwal P, Begom R, Rastogi SS, Sachan DS. A randomized, double blind, placebo controlled trial of L-carnitine in suspected acute myocardial infarction. *Postgrad Med* 1996;72:45-50.
- [6] Kukreja RC, Ockaili R, Salloum F and Xi, L. Cardioprotection with phosphodiesterase-5 inhibition-a novel preconditioning strategy. *J Mol Cell Cardiol* 2004;36:165-173.
- [7] Kharbanda RK, Peters M,\* Walton B, Kattenhorn M, Mullen M, Klein N, Vallance P, Deanfield J, MacAllister R.\* Ischemic preconditioning prevents endothelial injury and systemic neutrophil activation during ischemia-reperfusion in humans *in vivo*. *Circulation* 2001; 103:1624-30.
- [8] Broadhead MW, Kharbanda RK, Peters M \*MacAllister RJ.\* K-ATP channels activation induces ischemic preconditioning of the endothelium in humans *in vivo*. *Circulation* 2004; 110:2077-2082.
- [9] Tomai F, Crea F, Gaspardone A, \*Gaspardone A, Versaci F, De Paulis R, Penta de Peppo A, Chiariello L, Gioffre PA. \*Ischemic preconditioning during coronary angioplasty is prevented by glibenclamide, a selective ATP-sensitive K<sup>+</sup> channel blocker. *Circulation* 1994;90:700-705.
- [10] Gori T, Sicuro S, Dragoni S, \*Donati G, Forconi S, Parker JD.\* Sildenafil prevents endothelial dysfunction induced by ischemia and reperfusion via opening of ATP sensitive K channels. A human *invivo* study. *Circulation* 2005; 111:742-6.
- [11] Katz SD, Balidamaj K, Homma S \*Wu H, Wang J, Maybaum S.\* Acute type 5 phosphodiesterase inhibition with sildenafil enhances flow-mediated vasodilation in patients with chronic heart failure. *J Am Coll Cardiol* 2000;36:845-851.
- [12] Halcox JPJ, Nour KRA, Seals G \*Mincemoyer RA, Waclawiw M, Rivera CE, Willie G, Ellahham S, Quyyumi AA.\* The effect of sildenafil on human vascular function, platelet activation and myocardial ischemia. *J Am Coll Cardiol* 2002;40:1232-1240.
- [13] Yellon DM, Downey JM. Preconditioning the myocardium: from cellular physiology to clinical cardiology. *Physiol Rev* 2003;83:1113-1151.
- [14] Kloner RA. Cardiovascular effects of the 3phosphodiesterase-5 inhibitors approved for the treatment of erectile dysfunction. *Circulation* 2004; 110:3149-3155.
- [15] Reffelman T, Kloner RA. Effects of sildenafil on myocardial infarct size, microvascular function and acute ischemic left ventricular dilation. *Cardiovasc Res* 2003;59:441-449.
- [16] Mittleman MA, Glasser DB, Orazem J. Clinical trials of sildenafil citrate (Viagra) demonstrate no increase in risk of myocardial infarction and cardiovascular death compared with placebo. *Int J Clin Pract*. 2003 Sep;57(7):597-600.
- [17] Ockaili R, Salloum F, Hawkins J and Kukreja RC. Sildenafil(viagra) induces powerful cardioprotective

- effect via opening of mitochondrial K ATP channels in rabbits. *Am J Physiol* 2002, 283:H1263-H1269.
- [18] Das A, Ockaili R, Salloum and Kukreja, R.C. Protein kinase C plays an essential role in sildenafil-induced cardioprotection in rabbits. *Am J Physiol* 2003, 286:H1455-H1460.
- [19] Salloum F, Yin C, Xi L and Kukreja RC. Sildenafil induces delayed preconditioning through inducible nitric oxide synthase dependent pathway in mouse heart. *Circ Res* 2003;92:595-597.
- [20] Nagi O, Hajnal A, Parratt JR and Vegh A. Sildenafil(viagra)reduces arrhythmias severity during ischemia 24h after oral administration. *Brit J Pharmacol* 2004,141:549-551.
- [21] Swissa M, Ohara T, Lee MH, Kaul S, Shah PK, Hayashi H, Chen PS, Karagueuzian HS. Sildenafil-nitric oxide donor combination promotes ventricular tachyarrhythmias in the swine right ventricle. *Am J Physiol* 2001, 282:H1787-H1792.



## ARTICLE

# Aspirin Resistance: Need for a Specific, Rapid, Point-of-Care Assay

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## Abstract

### *Background*

Aspirin as a therapeutic drug has been in use for over hundred years. Its use as an anti-platelet drug was recognized in the early 70s. For secondary prophylaxis of vascular disease, it is the most useful and cost-effective drug. Large numbers of clinical trials using aspirin have concluded that at any given vascular risk, a low to medium dose of aspirin (80-160mg), is as effective as any other drug of choice. However, in recent years, there are several studies, in which aspirin resistance in patients with various vascular diseases have been demonstrated. Furthermore, some studies have suggested worst outcome in aspirin non-responders.

### *Methods*

A variety of methods have been used in these studies, to determine “non-responders” to the action of aspirin. The definition of “aspirin resistant” individuals is those whose platelet cyclooxygenase are not inhibitable by aspirin. Methods used for monitoring aspirin resistance in these studies are non-specific platelet function tests.

### *Results*

Differences in the definition of aspirin resistance, variations in the methods used to detect “non-responders”, inter-individual variations in the response of platelets to the action of different agonists, and lack of data from large clinical trials have hampered the advancement of knowledge in this area.

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## Conclusion

Therefore, there is a great need to develop specific and rapid Point-of-Care assays that could detect the prevalence of aspirin resistance in patient populations, so that those found to be non-responders to the action of aspirin might be provided alternate treatment regimen.

**Key words:** aspirin resistance, non-responders, thrombosis, stroke.

## Introduction

Role of platelets in the pathogenesis of atherosclerosis, thrombosis and stroke is well documented [1]. Therefore, there is a great need for developing specific and effective drugs for modulating platelet function. A thorough understanding of the signaling mechanisms involved in the regulation of platelet function will facilitate the development of better anti-platelet drugs. Agonists interact with the platelet at specific receptor sites on the plasma membrane and initiate a series of signaling events capable of modulating shape change, adhesion, aggregation, secretion of granule contents and expression of activation markers on the membrane [2-10]. Platelet aggregates are formed when the GP11b/111a receptors get activated and bind fibrinogen and recruit other platelets to form clumps. This phenomenon plays a significant role in the formation of effective haemostatic plug as well as growth of thrombus. Weak agonists such as epinephrine, ADP require the production of pro-aggregatory prostaglandin endoperoxides and thromboxane to cause platelet aggregation and secretion. Aspirin is a specific inhibitor of cyclooxygenase and prevents the formation of pro-aggregatory PG endoperoxides.

Data from a large number of clinical studies have demonstrated that at any given risk, irrespective of the disease state, aspirin at low to medium concentration is as effective as any other drug [2-5]. Although the ability of a plant bark (bark of willow, *Salix alba*) product to reduce fever was discovered two hundred years ago, the mechanism of action of aspirin remained elusive till late 1900 [6]. Nobel laureate Sir John R. Vane and his associates at the Royal College of Surgeons, in London, in 1971 proposed the first

satisfactory mechanism as to how aspirin works [7, 8]. Within a short period of time extensive work was done by various groups to elucidate the mechanism of action of aspirin like compounds [8-20]. During the same period, another Nobel Laureate Dr. Bengt Samuelsson of the Karolinska Institute, Stockholm, Sweden, discovered that the prostaglandin synthase produces transient bioactive prostanoids like PGG<sub>2</sub>/PGH<sub>2</sub> and thromboxane A<sub>2</sub> from the substrate arachidonic acid [10]. These findings revolutionized the research in platelet physiology and pharmacology [2-21].

## Platelet Physiology

Blood platelets interact with a variety of soluble agonists such as epinephrine (EPI), adenosine diphosphate (ADP), thrombin and thromboxane (TXA<sub>2</sub>); many cell matrix components, including collagen, laminin, fibronectin, and von Willebrand factor and biomaterials are used for construction of invasive medical devices [21-29]. These interactions stimulate specific receptors and glycoprotein-rich domains (integrin and non-integrin receptors) on the plasma membrane and lead to the activation of intracellular effector enzymes. Agonist-mediated activation of platelets stimulates phospholipase C (PLC) and it then triggers the hydrolysis of Phosphatidyl inositol 4, 5-bisphosphate, the formation of second messengers such as 1, 2-diacyl glycerol and inositol 1, 4, 5 trisphosphate. Diacylglycerol activates protein kinase and inositol trisphosphate facilitates the mobilization of free calcium from the storage sites. The majority of regulatory events appear to require free calcium. Ionized calcium is the primary bioregulator, and a variety of biochemical mechanisms modulate the availability of free calcium [27]. Elevation of cytosolic calcium stimulates phospholipase A<sub>2</sub> and liberates arachidonic acid (AA). Free AA is transformed to a novel metabolite thromboxane, a potent platelet agonist. This is the major metabolite of AA metabolism and plays an important role in platelet recruitment, granule mobilization, secretion of granule contents, and expression of activated GP11b/111a ( $\alpha_{11b}$   $\beta_3$ ) receptors [22-30]. Up regulation of activation signaling pathways, will increase the risk for clinical

complications associated with thromboembolic episodes.

## Arachidonic Acid Metabolism

Arachidonic acid is a 20 carbon polyunsaturated fatty acid (20:4w6) found in membrane phospholipids. Cell activation stimulates Phospholipase A<sub>2</sub>, which facilitates the release of this fatty acid from phospholipids. AA is converted to prostaglandin (PG) endoperoxides (PGG<sub>2</sub>/PGH<sub>2</sub>) by cyclooxygenase (Prostaglandin G/H synthase; COX1). These transient metabolites are converted by thromboxane synthetase to thromboxane A<sub>2</sub>, which is the major metabolite of this pathway in platelets [10]. Whereas, in vascular tissues, the endoperoxides generated by COX1 are transformed by prostacyclin synthetase to prostacyclin (PGI<sub>2</sub>). Thromboxane is a potent platelet agonist and a vasoconstrictor. Prostacyclin is an antiplatelet compound and exerts vasodilatory effects on vascular tissues. Thus from a single substrate (AA), two pharmacologically opposing vasoactive prostanoids are generated by platelets and vascular tissues [3]. Aspirin selectively acetylates the hydroxyl groups of a single serine residue (position 529) in the prostaglandin G/H synthase and causes irreversible inhibition of the activity of this enzyme [12, 13]. Inhibition of PG synthase results in the decreased conversion of AA to PG endoperoxides, PGG<sub>2</sub>/PGH<sub>2</sub>. Molecular mechanisms involved in aspirin-mediated inhibition of prostaglandin G/H synthase are well documented [4-16].

However, little is known about how other nonsteroidal anti-inflammatory drugs inhibit this enzyme [14]. To elucidate the mechanism by which this enzyme oxidizes AA, we developed a cell free assay system. This system used ferrous iron induced oxidation of AA. Oxidation of the fatty acids by iron was followed by nitrobluetetrazolium [31]. Several drugs known to be inhibitors of this enzyme were used in this system to evaluate the affinity of these compounds to ferrous iron. Results demonstrated these compounds could form a complex with ferrous iron and inhibit AA metabolism [32, 33]. Using a ferrous iron chelator, bipyridyl, we demonstrated the ability of this compound to inhibit platelet

prostaglandin synthesis and function [34]. In a separate study, we showed ibuprofen, a short acting drug, interacted with the active site of PG synthase and blocked the ability of aspirin to acetylate COX1 enzyme [35]. Based on these results and other studies, we concluded aspirin like compounds devoid of acetyl group interact with the heme part of the PG synthase and block AA oxidation [31-33]. In platelets AA is converted by lipoxygenase to 12-hydroxyeicosatetraenoic acid. This pathway is not inhibited by aspirin.

## Studies on the Use of Aspirin as an Inhibitor of Cox-1 Enzymes

Single oral doses of 10-100 mgs of aspirin can significantly inhibit the platelet PG synthase activity [36]. The inhibitory effect of aspirin on circulating platelets in the blood is fast and probably occurs in the portal circulation. The half-life of aspirin is very short (15-20 minutes) but sufficient to inhibit PG synthase of circulating platelets. Since these cells lack DNA and the ability to resynthesize the enzyme, the dysfunction caused by aspirin cannot be overcome. Therefore, platelets exposed to aspirin lose the ability to make the prostanoids completely. However, one should keep in mind that once the aspirin is hydrolyzed to salicylic acid, ability to inhibit prostaglandin synthase is lost. Hence the platelets produced from the marrow after the aspirin is hydrolyzed, will have active prostaglandin synthase. Approximately 10% of fresh platelets are added on to the circulating blood every day. Although aspirin treated blood platelets do not make prostaglandins, they respond with aggregation to the stimulation by prostaglandin endoperoxides and thromboxane. Fresh platelets formed after the hydrolysis of aspirin, can synthesize prostanoids and these newly formed metabolites of AA can cause aggregation of aspirin exposed platelets. In view of the fact that aspirin irreversibly inhibits prostaglandin synthase, it is possible to take advantage of repeated low-dose aspirin to achieve a cumulative effect [42-55]. Even doses as low as 30-50 mg aspirin taken daily will suppress platelet thromboxane synthesis significantly in 5 to 10 days.

Vascular tissues on the other hand have the ability to resynthesize prostaglandin G/H synthase [36]. Therefore, these cells can recover the enzyme activity following aspirin exposure. It is therefore, possible to develop a strategy to promote the biochemical selectivity of aspirin in terms of inhibition of platelet prostaglandin synthase. This is done by modification of the drug delivery, so the amount of drug delivered is just enough to inhibit platelet enzymes in the peripheral circulation and spare the systemic effect on vascular endothelium [37, 38]. Several studies have demonstrated the feasibility of this approach and various control release or timed release formulations have been developed for this novel therapy [37-39].

As mentioned earlier, aspirin is metabolized rapidly and the major metabolite, salicylic acid is a poor inhibitor of platelet prostaglandin synthase. Therefore, it is essential to develop appropriate strategies to maximize the beneficial effect of this novel drug. As low dose as 20 mg taken daily, reduces the platelet thromboxane formation by more than 90 percent. However, it is generally believed that higher doses are essential for preventing thromboxane dependent platelet activation. Studies by Wilson et al demonstrated that maximal plasma concentration of 12umol/L could be achieved by a single oral 50 mg dose of enteric-coated aspirin [17]. This dose was found sufficient to cause significant inhibition of platelet function and daily ingestion of low-dose aspirin demonstrated a cumulative effect. In a separate study, McLeod et al used doses ranging from 50-3900 mg of aspirin and monitored platelet function, bleeding time, and concluded that maximum dysfunction was obtained with daily doses of about 100 mg and no further changes were observed in these studies with higher doses [18]. Several workers have demonstrated the efficacy of low-dose oral aspirin in preventing platelet thromboxane production [2-4, 18, 44]. Indeed one of these studies has demonstrated beneficial effect of a dermal aspirin preparation on selective inhibition of platelet prostaglandin synthase, sparing the prostacyclin biosynthesis (38). It is very well established that 100 mg of aspirin per day is sufficient to significantly reduce the platelet thromboxane production [2-4, 20, 21, 40-42]. Furthermore, studies by McLeod et al have shown that dosages higher than 100 mg per day do not

produce any greater inhibition of platelet function or enhance bleeding times [18]. Therefore, it is reasonable to conclude that 80-160 mgs aspirin per day should be the choice for an ideal preventive protocol [41]. However, there is considerable room for improvement to maximize the benefits by better understanding the pharmacology of aspirin and platelet physiology [2-4]. It is possible to customize the aspirin treatment based on the individual patient needs. One can monitor the platelet prostaglandin synthase activity following aspirin ingestion and recommend a dose that is appropriate [34, 41]. It is possible to monitor the platelet response to agonists such as ADP or arachidonate and determine the degree of inhibition by aspirin like compounds [18]. In order to get maximum inhibition of platelet COX1 enzymes, continuous release aspirin formulations can be developed and tested against currently available aspirin formulations. Platelets are produced and released constantly to the circulation. Therefore, a time release aspirin which would make available small amounts of aspirin into the circulation may be effective. For instance, a 100 mg formulation capable of releasing 10 mg acetyl salicylic acid per hour may be better than a preparation which releases all of its active principle in a short span of time. Using the strategy of slowing down the release of active principle, newer formulations could be used effectively, to provide needed amounts of the drug into circulating blood at regular intervals. These novel formulations may also provide selectivity of aspirin action by preventing platelet thromboxane production and sparing the endothelial prostacyclin synthesis. McLeod et al studied the effect of various doses of aspirin (50, 100, 325, 1000 mg) on platelet and vascular tissues [43]. They did not observe inhibition of urinary 6-keto-PGF1 alpha production at low doses of 50 and 100 mg. They attributed these findings to the differential and selective inhibition of platelet function and the sparing effect of vascular COX1 enzymes. Sullivan and associates studied the effect of two different doses of aspirin on platelet function and TXA<sub>2</sub> production [45]. Platelet function in healthy volunteers was inhibited by both the doses (75 and 300 mg). Low dose failed to inhibit completely TXB<sub>2</sub> production 24 hours later, whereas 300 mg aspirin did. Even alternate day regimen of these doses prevented platelet function and significantly inhibited

the urinary levels of the 11-keto-TXB<sub>2</sub>. In a separate study, in healthy volunteers, formation of thrombin (Fibrinopeptide A; FPA), alpha granule release (beta-thromboglobulin; beta TG), and thromboxane (TXB<sub>2</sub>) were monitored in vivo, in blood emerging from a template bleeding incision [46]. At the site of plug formation significant platelet activation and thrombin generation was observed as indicated by 110 fold, 50 fold, and 30 fold increase in FPA, beta TG, and TXB<sub>2</sub>, within the first minute. A low dose regimen (0.42m/kg/day for 7 days) caused greater than 90% inhibition of TXB<sub>2</sub> formation in both bleeding time and clotted blood in these studies, suggesting critical role of platelet activation at the site of haemostatic plug formation. In a study to evaluate the effect of low dose aspirin (0.5 and 15 mg/kg/day) on platelet and renal prostanoids, Wilson et al monitored serum TXB<sub>2</sub> and urinary 6-keto PGF1 alpha [47]. Serum TXB<sub>2</sub> level was reduced to 3% of control by low dose and to 0.1% by the higher dose. Urinary TXB<sub>2</sub> was reduced only to 68% by low dose aspirin and to 51% by high dose. Urinary 6-keto-PGF1 alpha was not reduced by either dose. Based on their observation, they concluded low dose aspirin could significantly affect platelet PG production without affecting stimulated release of PGI<sub>2</sub> production.

## Clinical Studies on the Use of Aspirin

The two major clinical trials on aspirin concluded that ingestion of 160 mg per day or 325 mg alternative day provided significant benefit in preventing fatal events associated with CAD [20, 21]. Whereas, a 10 year trial involving nearly 40,000 women aged 45 and older with no evidence of cardiovascular disease found that a regular alternate day low-dose (100mg) aspirin was effective in reducing the incidence of stroke, but it did not have any effect on the incidence of heart attacks [48]. They concluded that the reasons for any sex-based differences in the efficacy of aspirin for primary prevention are unclear. According to Minnesota Heart Survey, about 6% of healthy women under age 65 and 30% of those over 65 take low-dose aspirin to prevent acute vascular events. Data from this primary prevention study dose not apply to women who already have had a heart attack or heart surgery or

diagnosed with coronary artery disease. For such women, as found in men, regular daily low dose (80-160mg) of aspirin clearly reduces the risk of developing acute coronary events.

Several earlier studies evaluated the effect of low dose aspirin on normal healthy volunteers as well as patients with various vascular diseases [45-58]. However, earlier studies did not report prevalence of any aspirin resistance. Zucker et al evaluated the effect of low dose aspirin (0.45mg/kg/day) and a high dose (900mg/day) in type 11 hyper-lipoproteinemic subjects [49]. They found that low dose aspirin effectively inhibited platelet function in these patients. Increased platelet thromboxane production has been described in several disorders including type-2 diabetes and type 11a hypercholesterolemia. This increased production of TXB<sub>2</sub> in hypercholesterolemic patients is attributed to abnormal cholesterol levels in these patients. It has been shown that even a low dose of aspirin (50 mg/7 days) significantly reduces 11-dehydro-TXB<sub>2</sub>, in these patients [50]. The effect of low dose aspirin has been evaluated in patients with diabetes, coronary heart disease, myocardial infarction (MI), cerebrovascular disease, peripheral artery disease and a variety of surgical procedures [45-58]. Diminno et al studied the effect of single doses of 100 and 1000 mg aspirin for 1 month in normal volunteers and patients with diabetic angiopathy [51]. They found a dose schedule of aspirin, which may suffice in normal volunteers, was not effective in patients with diabetic angiopathy. Contrary to this observation, Terres et al found a low dose of aspirin (100mg) caused significant inhibition of platelet function in both healthy subjects and patients with coronary heart disease [52]. Similarly, a low dose (0.45mg/kg/day) was found adequate for selective inhibition of TXA<sub>2</sub>-related platelet function, in patients recovering from MI [53]. Looks like the results on the effect of low-dose aspirin vary considerably, depending upon the type and stage of disease, dose of aspirin, and severity of procedure. In a study evaluating the effect of low dose aspirin (100mg) on hematological activity of left ventricular (LV) thrombus in anterior wall acute MI (AMI), Kupper et al found that low-dose had no effect on the incidence of hematologic activity and embolic potential of LV thrombosis in anterior wall AMI [54]. On the other hand, a low dose aspirin (40mg/day)

taken daily was found to be as effective as higher doses in preventing platelet functional responses in patients who had recent cerebral ischemia [55]. Uchiyama et al evaluated the effect of low dose aspirin, ticlopidine, and a combination of both these drugs in patients with cerebral ischemia [56]. Aspirin alone markedly inhibited platelet aggregation induced by AA, partially inhibited aggregation induced by ADP and did not inhibit aggregation by platelet activating factor. Combination of these drugs inhibited aggregation by all agonists. Rao et al demonstrated, in healthy volunteers, low doses of aspirin (40-80mg) had no inhibitory effect on the response of platelets to ADP, epinephrine and thrombin, but effectively inhibited the platelet response to threshold concentrations of AA [3, 4]. Epinephrine at concentrations too low to cause aggregation restored the sensitivity of aspirin-treated platelets to AA [59-61]. This phenomenon, in which weak agonists restore the sensitivity of drug-induced refractory platelets to the action of other agonists, was described from our laboratory as "mechanism of membrane modulation" [58-65].

## Aspirin Resistance

Studies from our laboratory for the first time demonstrated that one could induce drug-mediated resistance in platelets to the action of aspirin [66]. In this study, the subjects were given a short acting inhibitor of COX1, Ibuprofen. This was followed by administration of a full strength (325mg) aspirin. Ibuprofen-mediated inhibition of COX1 enzyme lasts for a short time, whereas, aspirin induced inhibition is irreversible. Ibuprofen treated platelets recovered their sensitivity to the action of AA by 24 hrs. Whereas, aspirin treated platelets failed to respond to the action of AA even after 24 hrs. In those subjects who had ingested aspirin after taking Ibuprofen first, aspirin failed to inhibit irreversibly the COX1; suggesting Ibuprofen molecules effectively prevented the acetylation of COX1enzyme by aspirin. One of the earliest work describing "non-responders" and "responders" evaluated the effect of low dose aspirin and a thromboxane synthetase inhibitor dazoxiben (UK3724B) in healthy subjects [57]. These studies demonstrated that low dose aspirin and ingestion of

two dazoxiben tablets prevented the release of granules from platelets in response to AA in some individuals (responders) and not in others (non-responders). These subtle differences in response of platelets to various drugs as well as differences in response to various agonists may be critical when considering the outcome of acute vascular events. For instance, collagen seems to exert its effect by multiple mechanisms. In a study, using aspirin, monoclonal antibodies to 11b-111a receptor and fibrinogen, it was demonstrated that there exists at least three mechanisms by which collagen activates platelets; 1) GP11b-111a associated activation, 2) prostaglandin dependent pathway, 3) alternate pathway responsible for 20-30% platelet aggregation [67].

Although it is well known that there are individual differences in response to drugs as well as agonists, this subject has not been studied thoroughly. Studies from Harvard researchers have demonstrated that the onset of myocardial infarction and sudden death is frequently triggered in early hours of the day. Aspirin and beta adrenergic blocking agents have been shown to prevent morning onset of acute vascular events [68]. In a limited chronobiologic study (n=7) on the efficacy of low-dose aspirin, international womb-to-tomb chronome initiative group demonstrated, that aspirin was more effective in the early hours of the day than late in the afternoon or evening [69, 70] Influence of dietary components and supplements on the efficacy of anti-platelet drugs also is poorly understood. Vitamin E and its quinone are potent inhibitors of platelet function and interfere with the release of arachidonic acid by cell membranes [71, 72]. Studies from our laboratory have demonstrated that heme chelators, non-steroid anti-inflammatory drugs, antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole and fish lipids compete with arachidonic acid metabolism and prevent the formation of pro-aggregatory PG metabolites [71-74]. Docosahexaenoic acid (DHA) is poorly metabolized by Cox-1 enzymes. However, *in vitro* studies have demonstrated that DHA is a potent inhibitor of AA metabolism and platelet function [74]. Eicosapentaenoic acid, oleic acid, linoleic acid, linolenic acid competitively inhibit COX1 enzymes in *in vitro* studies. We do not know very much about the influence of dietary lipids on AA metabolism by platelets.

Several recent studies have demonstrated drug resistance in patients with a variety of vascular diseases [75-118]. This subject currently is a very hot topic and has made national headlines. Andrew Pollack published an article in July of 2004 in New York Times, on this subject titled, "For Some, Aspirin May Not Help Hearts" [75]. According to this article, 5-40% of aspirin users are "non-responders" or "resistant" to the drug. In the same article, he cites the opinion of Dr. Daniel I. Simon, the associate director of interventional cardiology at Brigham and Women's Hospital, Boston, which reads as follows: "They are taking it for stroke and heart attack prevention and it's not going to work". He also reports the opinion of Dr. Michael J. Domanski; head of clinical trials unit at the NIH, in his opinion, the non-responders may represent a huge number of patients. According to Dr. Deepak L. Bhatt, director of interventional cardiology Cleveland Clinic, aspirin resistance is associated with worst outcome. Professor Eric Topol, Chairman, Cardiovascular Medicine Cleveland Clinic, USA states, "Aspirin resistance carries high risk, with over 20 million Americans taking aspirin to prevent heart attacks or strokes, it is important that further work to be done to confirm our findings and develop a rapid detection method. He also assures that for individuals with aspirin resistance, there are excellent alternatives".

These observances from health care providers and researchers raise a number of issues. Do we know enough about aspirin resistance? What is the prevalence of aspirin resistance in the healthy population? What causes this resistance to develop in patient populations? Are there specific, rapid, cost-effective tests available? What alternative long-term treatments are available, if patients are resistant to common anti-platelet drugs such as aspirin and Clopidogrel? Should the doses of these drugs used for therapy be increased? Should we drop the use of these drugs in non-responders? We need to find answers to these and other emerging questions soon. In the next few paragraphs a brief overview of what is known about the prevalence of aspirin resistance, clinical findings, and methodologies available, will be provided.

The first and foremost need at this time is to standardize a definition of aspirin resistance. The mechanism of action of aspirin is very well

documented [7-13]. The drug acetylates the platelet COX1 enzyme and irreversibly inhibits its ability to convert AA to PG endoperoxides [12, 13]. In the absence of COX1 enzyme activity, platelets do not respond to AA stimulation with aggregation. Weak agonists such as ADP, Epinephrine depend on the formation of PG endoperoxides to initiate a secondary wave of aggregation and promote release of platelet granule contents [3]. Therefore, weak agonists fail to induce platelet aggregation and release granules from aspirin-treated platelets. Failure of AA, ADP and Epinephrine to cause aggregation of platelets more or less establishes drug-induced platelet dysfunction. If platelets obtained from individuals who have ingested a full strength aspirin, respond with aggregation to the action AA, ADP and EPI, and release their granule contents, then one can safely conclude that these platelets are resistant to aspirin action. Further proof for aspirin resistance of platelets can be provided by studying AA metabolism by such platelets, monitoring serum TXB<sub>2</sub> levels, or urinary levels of TXB<sub>2</sub> or its metabolite, 11-dehydro-TXB<sub>2</sub>. Methods are available to monitor all these parameters. According to Cattaneo, "aspirin resistant" should be considered as a description for those individuals whom aspirin fails to inhibit thromboxane A2 production, irrespective of the results of unspecific tests of platelet function [110].

## Prevalence of Aspirin Resistance

Aspirin resistance has been poorly defined, variety of non-specific methods have been employed to monitor the "aspirin resistance" and conflicting reports have been published on the rates of prevalence and outcome of continuing this therapeutic modality [75-93]. Aspirin resistance has been reported in patients with cardiovascular, cerebrovascular, and peripheral vascular disease [75-93]. Because of the differences in methodologies used to monitor this phenomenon and lack of a specific assay to determine the true aspirin resistance, there is considerable confusion and the true significance of this observation remains obscure [76- 78]. It also raises the question, as to how we missed this phenomenon of drug resistance all these years? Large numbers of clinical trials have demonstrated the beneficial effects of

aspirin therapy irrespective of the disease state [40]. Is it possible that these earlier trials missed aspirin non-responders? On the other hand, it is quite possible that only responders to the action of aspirin received the benefit of this therapy.

Studies in our laboratory over three decades, have failed to show any aspirin resistance in normal healthy subjects. The only subject whose platelets failed to aggregate in response to AA stimulation was found to be deficient in platelet COX1 enzyme activity [60]. Platelets obtained from this subject responded with aggregation when stirred with epinephrine and arachidonate, suggesting PG endoperoxides and TXA<sub>2</sub> are not essential to cause irreversible aggregation of platelets. There is not much data on the prevalence of aspirin resistance in general healthy subjects. In patients with various vascular diseases, the rate of non-responders reported varies between less than 2% to over 60%. Since the methods used to monitor aspirin resistance in these reports are not specific, the prevalence rate published is debatable [75-93].

Hurlen et al used the method of Wu and Hoak to determine the platelet aggregation ratio as a marker for assessing platelet function and evaluated the effect of aspirin (160mg/day) in 143 patients who had survived myocardial infarction [80, 81]. Based on their definition of non-responders to the action of aspirin, they could only identify two subjects as primary non-responders. Gum et al from Cleveland Clinic studied 326 stable cardiovascular subjects on aspirin (325mg/day) and tested aspirin sensitivity by platelet response to aggregating agents such as ADP and AA. They found 5.5% as non-responders to aspirin and 24% as semi-responders [82]. Gum and associates used the PFA-100, a method that measures platelet function, to determine aspirin resistance in their patient population [83]. Based on the results of their studies with this methodology, they found 9.5% to be non-responders to aspirin action.

Some studies have reported as high as 30-40% non-responders of stroke or vascular disease patients and predicted >80% increase risk for a repeat event during a 2-year follow up period [84-88]. Eikelboom et al analyzed base line urinary levels of TXB2 metabolites 11-dehydro thromboxane B2 in 5529 patients enrolled in the Heart Outcomes Prevention Evaluation (HOPE) study [88]. Of these subjects 488

were on aspirin regimen. On the basis of their findings they concluded that in aspirin-treated patients, increased levels of urinary metabolite of TXB<sub>2</sub> predict future risk of myocardial infarction or cardiovascular death. The patients with the highest levels of urinary TXB<sub>2</sub> metabolite had 3-5 fold higher risk of cardiovascular death compared to those in the lowest quartile. Another study reporting clinical outcomes of aspirin resistance is from Austria [85, 87, 90]. In this study patients undergoing arterial angioplasty were on 100 mg aspirin per day. Platelet function was assessed by whole blood aggregometry. This study demonstrated that reocclusion at the sites of angioplasty occurred only in men for whom platelet dysfunction was evident by aggregometry [87]. Zimmerman et al identified aspirin non-responders as those who had >90% inhibition of TXB<sub>2</sub> formation in presence of 100umol/L aspirin and 1mmol/L arachidonate [91]. In patients who had undergone coronary bypass surgery (CABG), AA and Collagen stimulated formation of TXB<sub>2</sub> was the same before and after CABG, indicating oral aspirin did not significantly inhibit platelet COX1. However, the *in vitro* studies with 100umol/L aspirin on blood obtained from these subjects showed decreased TXB<sub>2</sub> (>10%) in most samples studied. They concluded that platelet COX1 inhibition by aspirin is compromised for several days after CABG, probably due to an impaired interaction between aspirin and platelet COX1. This observation indicates how complex the issues are when evaluating the effect of anti-platelet drugs during and after interventional procedures. Sane et al evaluated the effect of aspirin (325 mg/day/month) in patients suffering from congestive heart failure [92]. These researchers used whole blood aggregometry (Chronolog Corp, PA., USA), Platelet receptor expression by flow cytometry and PFA-100. Patients were considered non-responders when 4 of the 5 parameters assayed were observed. Using this complex rating, persistent platelet activation was observed in 50 of the 88 patients (56.8%). These observations remind us of the inadequacy of the existing methods to detect what truly represents "aspirin resistance".

## Clopidogrel Resistance

Muller et al have reported the prevalence of Clopidogrel "non-responders" among patients with stable angina scheduled for coronary stent replacement [93]. They evaluated the effect of 100mg/day aspirin with a loading dose of 600mg of Clopidogrel. They defined Clopidogrel non-responders by an inhibition of ADP (5and 30umol/L) induced platelet aggregation to less than 10% of base line values after the ingestion of the drug. They found 5-11% of the patients as non-responders and 9-26% semi-responders. Lau et al studied both normal subjects and patients undergoing stent replacement to determine the prevalence of drug resistance [94]. Since Clopidogrel is activated by hepatic cytochrome P450 (CYP) 3A4, they monitored the activity of this enzyme in both test subjects. They concluded that some patients are resistant to these drugs because of biological variability and drug-drug interactions. They also attributed the difference due to variations in (CYP) 3A4 activity. Angiolillo et al compared the effect of standard dose of 300mg with a high dose of 600mg Clopidogrel after coronary stenting [95]. They concluded that use of higher dose optimizes platelet inhibitory effects. Serebruany et al evaluated the effect of Clopidogrel in a large heterogenous population using ex vivo measurements of platelet aggregation [96]. Prevalence of hypo-responsiveness and hyper-responsiveness to Clopidogrel in these patient population was 4.2% and 4.8% respectively. A Polish study with a small sample size (n=31) studied the effect of Clopidogrel (300) in patients with stable angina using a rapid whole blood platelet function assessment [97]. They conclude that the clinical importance of Clopidogrel resistance is still debatable. In view of the fact that data are not available on large number of clinical trials, their conclusion is reasonable.

## Methodologies

Researchers have used a variety of methods to assess the aspirin and Clopidogrel resistance [81-98]. In spite of the inherent problems associated with platelet aggregometry, it still is the gold standard to detect aspirin sensitivity of platelets. Studies from our

laboratory and that of others have shown even low doses (40-80 mg) of aspirin effectively inhibit arachidonate-mediated platelet aggregation. In the absence of any other specific rapid Point-of-Care assay system for detecting the sensitivity of platelets to aspirin or lack of aspirin sensitivity, it is preferable to use optical whole blood aggregometry to validate the results obtained by other methods. Hurlen et al used platelet aggregate ratio (PAR) to evaluate the effect of aspirin in post-MI patients [80]. Basically the method developed by Wu and Hoak uses a ratio of platelets obtained with EDTA/formalin as anti-coagulants and comparing the counts of platelets obtained with EDTA [80, 81]. In the presence of platelet aggregates the ratio is <1.0. Using this method they found two individuals as primary non-responders and 14 patients as secondary non-responders.

Several researchers have used Platelet Function Analyzer PFA-100 (Dade International Inc, Miami USA), to evaluate aspirin resistance in patients with various vascular diseases [83, 92, 101, 103, 104, 113, 119]. This analyzer measures platelet function as their ability to occlude a membrane coated with collagen and epinephrine (EPI) or collagen and ADP under high sheer stress. Sambola et al evaluated the effect of low dose aspirin (100mg/day/1month, 6month) in 100 patients with acute coronary syndrome [101]. At one-month post-aspirin therapy, they found 49 patients to have sub-optimal response to aspirin (SASAR), whereas only 25 of them showed SASAR by conventional aggregometry. Anderson et al evaluated the effect of 75 mg aspirin in patients (n=202) with MI. In patients on aspirin alone they found 25/71 as non-responders with the epinephrine cartridge. They did not find a significant difference between EPI cartridge and ADP cartridge. Gum et al. evaluated the phenomenon of aspirin resistance in 325 stable cardiac patients [83]. They found 5 per cent were non-responders and 23 per cent semi-responders by optical aggregometry. Whereas, using PFA-100 they found 9.5% as aspirin resistant. This detection system does not seem to detect difference between the effects of low and high-dose aspirin on platelet function. Since it evaluates the synergistic effect of a combination of two agonists on platelet function, the results obtained or similar to that obtained in our aggregation studies [58-66]. In a series of studies we have demonstrated a

combination of two agonists overcomes the inhibition by aspirin and other drugs [58-66].

Malinin et al used THE Ultegra RPFA-ASA (VerifyNow; Accumetrics, Inc., San Diego, California, USA) to monitor the effect of a single dose of aspirin (325 mg) in subjects with multiple risk factors [100]. This system uses turbidometric optical detection system. It uses a test cartridge that contains lyophilized preparation of human fibrinogen-coated beads, platelet agonist, and buffer. Fibrinogen-coated beads bind available receptor on platelet membrane. A cationic agonist, propyl gallate, is used to obtain complete activation of platelets. It uses citrated whole blood for assays. The results are expressed in aspirin response units (ARU). Of the 14 subjects studied, only one was found to be non-responsive by aggregometry, whereas they found 10 subjects as non-responders by RPFA-ASA. They concluded this system was a sensitive device for measuring anti-platelet responsiveness. The newly introduced "VerifyNow" kit includes arachidonic acid as an agonist for activating platelets. Inclusion of this agonist instead of propyl gallate has considerably improved the performance of this system. In our hands, this system is as good as whole blood aggregometry (Chronolog Corporation) in identifying aspirin non-responders.

Aspirin resistance could be monitored by a variety of assays, including platelet aggregometry, PAR, PFA100, RPFA-ASA; plasma, serum, urinary TXB<sub>2</sub> and urinary 11-dehydrothromboxane-2 [46, 47, 80-84, 87, 88, 92, 95, 98, 100]. We use majority of these methods to validate data in our efforts to develop a rapid, specific Point-of-Care assay system, for monitoring platelet function. The majority of these methods are labor intensive, time consuming and expensive. Technologies are available to develop a rapid and specific assay system, which can monitor platelet function. Specific fluorescent antibodies can be used to detect platelet bound fibrinogen, P-selectin or released granule products such as beta-thromboglobulin, platelet factor 4 and soluble P-selectin. Furthermore, ATP release or thromboxane generated also could be easily monitored. All the available methods use large quantities of blood and take a considerable amount of time. It is possible to devise methods that use only small quantities of blood obtained from a finger stick. By using state-of-the-art

detectors one could detect the luminescence (ATP) or fluorescence (specific antibodies) in minutes and develop needed software to standardize the results. If the assay uses AA as a standard platelet agonist then specificity could be built into this system for monitoring aspirin sensitivity. Similarly by using ADP as an agonist one can build the needed specificity to monitor non-responders to Clopidogrel. The limiting factors for non-availability of such a Point-of-Care assay system are lack of funds and the assumption that currently available methods detect platelet sensitivity or otherwise to anti-platelet drugs effectively.

## Discussion

Aspirin is the most-effective, widely used, relatively inexpensive anti-platelet drug currently available. In the developing countries, aspirin is the most cost-effective drug for primary as well as secondary prophylaxis for platelet-mediated vascular complications [3-5]. The mechanism by which it inhibits platelet function is well documented [7-14]. Many large clinical trials have established beyond any doubt that aspirin therapy significantly reduces the incidence of acute events in a variety of vascular diseases [40]. Recent studies have provided evidence to suggest that some sub-populations of patients on aspirin therapy may have developed resistance to the action of aspirin [75-98]. These observations have raised an alarm to the public as to who is getting protection and who is not? Since there is no simple test that can be performed at the doctor's office to detect such a condition there is some panic in the patient population. Furthermore, there is still a lack of awareness of this problem in the medical community at the time of this writing. There are only two major studies, which have described adverse outcome in those patients who are defined as non-responders to the action of aspirin [83, 88].

Based on methods, which still are not very specific, researchers have identified a sub-population of patients who are considered non-responders and another group as semi-responders to the action of aspirin [82-98]. Although the majority of methods used for monitoring this phenomenon lack specificity, it is important to recognize that this problem exists. If

these patients for whatever reason are not getting appropriate protection from drug therapy, there is an immediate need to identify them and change their therapeutic regimen. It is equally important to recognize that even those who are considered responders, may need additional protection to prevent adverse outcomes, if they have hyper-responsive platelets or hyper-responsive coagulation pathways. Basically, we need to identify aspirin non-responders, individuals with hyperactive platelets/coagulation pathways.

The subject of aspirin resistance has received lot of attention in the press and scientific publications [75-98]. However, the practicing physicians and the public are not fully aware of the problem. Therefore, there is an immediate need for the development of awareness programs to educate the health care providers as well as the public who receive health care. Since there is considerable interest in this subject in the research community, a number of reviews have been published on this subject [75-98]. No attempt has been made in this article to extensively review this subject. Focus of this article has been to bring into discussion three specific areas of concern. First and foremost is the molecular mechanism involved in the initiation of thrombosis and stroke. Basically two major pathways (platelet activation dependent and coagulation cascade activation dependent) play a role in the pathophysiology of this process. Therefore, there is a need to identify the hyper-responsiveness of individuals to both these pathways and develop appropriate combination therapy. Secondly, the arachidonic acid pathway blocks only one of the many mechanisms modulating platelet activation. Therefore, there is an immediate need to develop appropriate drugs or drug combinations to prevent the common pathway of platelet activation. Since fibrinogen binding to activated GP 11b/11a receptor promotes thrombus formation and growth, drugs should be designed and developed to modulate these mechanisms. Third, there is a need for better Point-of-Care assays, which can profile the coagulation pathway as well as platelet activation mechanisms. Such an assay system could be effective in identifying non-responders to commonly used anti-platelet drugs, semi responders or hyper responsive individuals and those with hyper coagulable states. Since there is no

such detection system a lot of individuals at risk are not getting appropriate treatment or prophylaxis against acute vascular events.

In the early 80s, based on our extensive studies, we concluded that aspirin and other anti-platelet drugs do not prevent platelet activation under a variety of experimental conditions [58-66]. Aspirin only inhibits one of the many platelet activation mechanisms. Furthermore, it does not prevent platelet interaction with vessel wall [63]. However, in a large number of clinical studies, aspirin has been shown to offer significant beneficial effects to patients with various vascular diseases [40]. Several recent studies have demonstrated that there exists some degree of non-responders to the action of aspirin [75-98]. Therefore, it is of great importance to thoroughly examine this issue of drug resistance and determine the prevalence of true "aspirin resistance" among the patients and provide them alternate therapies. Since our studies have clearly demonstrated epinephrine, norepinephrine, and ADP can potentiate the action of other agonists on platelets, we may as well screen those who are hyper responsive to various agonists, so that these individuals also could be provided appropriate therapeutic regimen. There are reports suggesting those who are non-responders to the action of aspirin may be more responsive to the action of agonists such as ADP or collagen. Studies should take into consideration that platelets can be stimulated by a variety of soluble agonists as well as cell matrix components and increased shear stress.

In spite of the fact that several attempts have been made to explain the mechanisms involved in aspirin resistance, there exists no clear explanation. Studies should be initiated immediately to explore the mechanism by which an individual develops resistance to these drugs. Since platelets lack DNA, it is highly unlikely that these cells re-synthesize COX1 enzyme. Since many of these patients are on long-term aspirin therapy the effect of aspirin on megakaryocytes should be further explored. Platelets are formed from megakaryocytes and these cells do have DNA, hence studies are warranted to explore the effect of aspirin on bone marrow megakaryocytes. The majority of patients with vascular disease also will be taking many other drugs. As such, some amount of drug-drug interaction cannot be ruled out. Whatever may be the mechanism by which

individuals with vascular disease develop resistance to aspirin, a better understanding of the molecular mechanisms involved will facilitate the development of the appropriate therapeutic regimen.

Now that anti-platelet drug resistance has surfaced out and created concern in the clinical community, there will be renewed interest in the development for improving the available technologies for monitoring platelet and coagulation pathways [75-118]. Chronolog corporation, one of the leaders in developing optical aggregometers, has already come up with a less expensive whole-blood aggregometer for monitoring aspirin resistance. The accumetric system has recently introduced arachidonic acid as a stimulant instead of propyl gallate, for monitoring aspirin resistance. Similarly, research and development work is going to improve the PAF-100 system as well, for monitoring aspirin resistance. Currently available data on the use of PFA-100 to monitor aspirin resistance is inconclusive. A large study using 1000 patients is in progress to evaluate the applicability of PFA-100 to monitor drug resistance [119]. Results from this study may shed some light on the usefulness of this detection system. All the currently available methodologies require relatively large quantities of fresh blood. Platelet function assays are labor intensive, non-specific and time consuming. Future direction of research will focus on the development of rapid, specific, Point-of-Care assays using minimal amounts of blood from a finger stick, for monitoring platelet function as well as coagulation profile.

## Conclusion

Platelets play a very important role in the pathogenesis of atherosclerosis, thrombosis and stroke. Aspirin, even at a low to medium dose (80-160mg/day) has been shown to offer significant protection to individuals from developing acute vascular events. Aspirin is the most cost-effective drug available for the secondary prophylaxis of cardiovascular diseases. From the available evidence both experimental and clinical, it is reasonable to conclude, that a significant number of patients are not getting full protection from the use of aspirin. Even those who are considered responders to aspirin

therapy may need additional protection, if their platelets are hypersensitive or have a hyper-responsive coagulation pathway. Since currently available methods to assess aspirin sensitivity are not specific, labor intensive and time consuming; efforts should be made to develop specific, rapid, detection methods. Serious attempts should be made to develop state-of-the-art Point-of-Care assay systems, which are capable of detecting not only individuals who develop resistance to currently available anti-platelet drugs but also those, who are hyper-responsive to both platelet and coagulation activation mechanisms.

## References

- [1] Sherry S, Scriabine A. *Platelets and Thrombosis*. University Press, USA 1999. P.309
- [2] Sudow C, Baigent C. Randomized Trials of Antiplatelet Therapy. *Handbook of Platelet Physiology and Pharmacology*. Rao GHR, (ed) Kluwer Academic Publishers, USA1999, p526-549.
- [3] Rao GHR, Rao AT. Pharmacology of Platelet Inhibitory Drugs. *Ind. J. Physiol.* 1994, 38:69-84.
- [4] Rao GHR, Rao ASC, White JG. Aspirin in Ischemic Heart Disease-an overview. *Ind. Heart J.* 1993, 45: 73-79.
- [5] Rao GHR. Aspirin and Coronary Artery Disease: Coronary Artery Disease in South Asians: Epidemiology, Risk Factors and Prevention. Rao GHR, Kakkar VV(eds), *Jaypee Medical Publishers*, India, 2001, p263-278.
- [6] Weisman G. Aspirin. *Sci Am.* 1991, 264: 84-91.
- [7] Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action of aspirin-like drugs. *Nature* 1971, 231: 232-235.
- [8] Vane JR, Flower RJ, Botting RM. History of aspirin and its mechanism of action. *Stroke* 1990, 21: IV-12.
- [9] Ferreira SH, Vane JR. Newer aspects of the mode of action of non-steroidal anti-inflammatory drugs. *Ann. Rev. Pharmacol.* 1974, 14: 57-73.
- [10] Hamberg M, Svensson J Samuelsson B. Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc. Natl. Acad. Sci.* 1975, 72:2994-2998.
- [11] Marcus AJ. Aspirin as an anti-thrombotic medication. *N. Engl. J. Med.* 1983, 309: 1515-1517.
- [12] Roth JG, Caverley DC. Aspirin, Platelets and Thrombosis: Theory and Practice. *Blood* 1994, 83: 885-898.
- [13] Roth JG: Stanford N, Majerus PW. Acetylation of prostaglandin synthetase by aspirin. *Proc. Natl. Acad. Sci.* 1975, 72: 3073-3076.

- [14] Meade EA, Smith WL, Dewitt DL. Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isoenzymes by aspirin and other non-steroidal anti-inflammatory drugs. *J. Biol. Chem.* 1993, 268: 6610-6614.
- [15] Burch JW, Stanford N, Majerus PW. Inhibition of platelets prostaglandin synthetase by oral aspirin. *J. Clin. Invest.* 1978; 61: 314-319.
- [16] Reilly IA, FitzGerald GA. Aspirin in Cardiovascular Disease. *Drugs* 1988, 35: 154-176.
- [17] Wilson KM, Siebert DM, Duncan EM. et al. Effect of aspirin infusions on platelet function in humans. *Clin. Sci.* 1990, 79: 37-42.
- [18] McLeod LJ, Roberts MS, Cossum PA. et al. The effects of different doses of some acetyl salicylic acid formulations on platelet function and bleeding times in healthy subjects. *J. Haematol.* 1986, 36: 379-384.
- [19] Masptti G, Galanti G, Pogessi L. Differential inhibition of prostacyclin production and platelet aggregation by aspirin. *Lancet* 1972, 2:11213-1216.
- [20] Steering Committee of the Physicians Health Study Research Group. Preliminary Report: Findings from the aspirin component of the ongoing physicians' health study. *N. Engl. J. Med.* 1988, 318: 262-264.
- [21] Steering Committee of the Physicians Health Study Research Group. Final Report. *N. Engl. J. Med.* 1989, 321: 129-135.
- [22] Hallam TJ, Sanchez A, Rink TJ. Stimulus response coupling in Platelets. *Biochem J.* 1984, 218:819-827.
- [23] Zucker MB Nachmias VT. Platelet Activation. *Arteriosclerosis*. 1985, 5:2-18.
- [24] Edridge MJ. The molecular basis of communication within the cells. *Sci. Am* 1985, 253:142-152.
- [25] Holmsen H. Platelet metabolism and activation. *Semin Hematol.* 1985, 22:219-240.
- [26] Seiss W. Molecular mechanism of platelet activation. *Physiol. Rev.* 1990, 70:115-164.
- [27] Rao, GHR. Physiology of blood platelet activation. *Ind J Physiol Pharmacol.* 1993, 37:263-275.
- [28] Rao, GHR. Signal transduction, second messengers and platelet function. *J Lab Clin Med.* 1993, 121:18-21.
- [29] Rao, GHR. Signal transduction, second messengers and platelet pharmacology. *Pharmacol.* 1994,13:39-44.
- [30] Packham MA. Role of platelets in thrombosis and hemostasis. *Can J Physiol. Pharmacol.* 1993, 72:278-284.
- [31] Rao GHR, Gerrard JM, Eaton JW, White JG. The role of iron in prostaglandin synthesis: Ferrous iron mediated oxidation of arachidonic acid. *Prost and Med* 1978, 1: 55-70.
- [32] Peterson DA, Gerrard JM, Rao GHR, Mills EL, White JG. Interaction of arachidonic acid and heme iron in the synthesis of prostaglandins. *Adv Prost and Thromb Res* 1980, 6; 157-161.
- [33] Peterson DA, Gerrard JM, Rao GHR, White JG. Inhibition of ferrous iron induced oxidation of arachidonic acid by indomethacin. *Prost and Med* 1979, 2: 97-108.
- [34] Rao GHR, Cox AC, Gerrard JM, White JG. Effects of 2,2' dipyradil and related compounds on platelet prostaglandin synthesis and platelet function. *Biochem Biophys Acta* 1980, 628: 468-479.
- [35] Rao GHR, Johnson GJ, Reddy KR. Ibuprofen protects cyclooxygenase from irreversible inhibition by aspirin. *Arteriosclerosis* 1983, 3: 384-388.
- [36] Patrono C. Aspirin as an antiplatelet drug. *N Engl. J Med* 1994, 330: 1287-1294.
- [37] Hanley SP, Cockbill SR, Bevan J. et al. Differential inhibition by low-dose aspirin of human venous prostacyclin synthesis and platelet thromboxane synthesis. *Lancet* 1981, 2: 969-971.
- [38] Keimowitz RM, Pulvermacher G, Mayo G. et al. Transdermal modification of platelet function: A dermal aspirin preparation selectively inhibits platelet cyclooxygenase and preserves prostacyclin biosynthesis. *Circ* 1993, 88: 556-561.
- [39] Clarke RJ, Mayo G, Price P. et al. Suppression of thromboxane A<sub>2</sub> but not systemic prostacyclin by controlled release aspirin. *N Engl. J Med* 1991, 325: 1137-1141.
- [40] Antiplatelet Trialists' Collaboration. The Aspirin Papers. *Brit. J. Med.* 1994, 308:71-72, 81-106.
- [41] Fuster V, Dyken ML, Vokomas PS. Aspirin as a therapeutic agent in cardiovascular disease. *Circ.* 1993, 87:659-675.
- [42] Rao GHR, Reddy KR, White JG. Low-dose aspirin, platelet function and prostaglandin synthesis: influence of epinephrine and alpha-adrenergic receptor blockade. *Prost. and Med.* 1981, 6:485-494.
- [43] McLeod LJ, Roberts MS, Seville PR. Selective inhibition of platelet cyclooxygenase with controlled release low dose aspirin. *Aust. N Z J Med.* 1990, 20: 652-656.
- [44] Rao GHR, Radha E Johnson GJ. et al. Enteric coated aspirin, platelet cyclooxygenase activity and platelet function. *Prost. Leukot. Med.* 1984,13: 3-12.
- [45] Sullivan MH, Zosmer A, Gleeson RP. et al. Equivalent inhibition of *in vivo* platelet function by low dose and high dose aspirin. *Prost. Leukot. Fatty Acids.* 1990,39:319-321.
- [46] Kyrle PA, Eichler HG, Jager U. et al. Inhibition of prostacyclin and thromboxane A<sub>2</sub> generation by low dose aspirin at the site of plug formation in man *in vivo*. *Circ.* 1987, 75:1025-1029.
- [47] Wilson TW, McCauley FA, Wells HD. Effects of low dose aspirin on responses to Furosemide. *J. Clin. Pharmacol.* 1986, 26:100-105.
- [48] Ridker PM, Cook NR, Lee IM, et al. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women. *N. Engl.J. Med.* 2005, 352, 1293-304.

- [49] Zucker MI, Trowbridge C, Woodroof J. et al. Low- vs high-dose aspirin. Effects on platelet function in hyperlipoproteinemic and normal subjects. *Arch. Intern. Med.* 1986, 146: 921-925.
- [50] Davi G, Averna M, Catalano I. et al. Increased thromboxane biosynthesis in type 11a hypercholesterolemia. *Circ.* 1992, 85: 1792-1798.
- [51] Diminno G, Silver MJ, Cerbone AM et al. Trial of repeated low-dose aspirin in diabetic angiopathy. *Blood.* 1986, 68: 886-891.
- [52] Terres W, Schuster O, Kupper W. et al. Effects of low-dose acetylsalicylic acid on thrombocytes in healthy subjects and in patients with coronary heart disease. *Dtsch Med. Wochenschr* 1989, 18: 1231-1236.
- [53] De Caterina R, Giannessi D Bernini W. et al. Low-dose aspirin in patients recovering from myocardial infarction. Evidence for a selective inhibition of thromboxane-related platelet function. *Eur. Heart J.* 1985, 6:409-417.
- [54] Kupper AJ, Verheugt FW, Peels CH. et al. Effect of low dose acetylsalicylic acid on the frequency and hematologic activity of left ventricular thrombus in anterior wall acute myocardial infarction. *Am. J. Cardiol.* 1989, 63:917-920.
- [55] Weksler BB, Kent JL, Rudolph D. et al. Effects of low dose aspirin on platelet function in patients with recent cerebral ischemia. *Stroke.* 1985, 16: 5-9.
- [56] Uchiyama S, Sone R, Nagayama T et al. Combination therapy with low-dose aspirin and ticlopidine in cerebral ischemia. *Stroke.* 1989, 20: 1643-1647.
- [57] Jones EW, Cockbill SR, Cowley AJ. et al. Effects of dazoxiben and low-dose aspirin on platelet behavior in man. *Brit. J. Pharmacol.* 1983, 15: 395-445.
- [58] Rao GHR, White JG. Epinephrine-induced Platelet Membrane Modulation. The Platelet Amine Storage. Myers KM, Barnes CD (eds) *CRC Press*, Roca Baton, USA.1992, p117-149.
- [59] Rao GHR, Johnson GJ, White JG. Influence of epinephrine on the aggregation response of aspirin-treated platelets. *Prost. Med.* 1980, 5:45-58.
- [60] Rao GHR, White JG. Epinephrine potentiation of arachidonate-induced aggregation of cyclooxygenase deficient platelets. *Am. J. Hematol.* 1981, 11: 355-366.
- [61] Rao GHR, White JG. Role of arachidonic acid in human platelet activation and irreversible aggregation. *Am. J. Hematol.* 1985, 19: 339-347.
- [62] Rao GHR, White JG. Aspirin, PGE1 and Quin-2 AM induced platelet dysfunction. Restoration of function by nor-epinephrine. *Prost. Leukot. Essent. Fatty Acids* 1990, 39: 141-146.
- [63] Rao GHR, Escobar G, White JG. Epinephrine reverses the inhibitory influence of aspirin on platelet vessel wall interaction. *Thromb. Res.* 1986, 44: 65-74.
- [64] Rao GHR, Escobar G, Zavrol J. Influence of adrenergic receptor blockade on aspirin-induced inhibition of platelet function. *Platelets* 1990, 1:145-150.
- [65] Rao GHR, Reddy KR, White JG. Modification of human platelet response to sodium arachidonate by membrane modulation. *Prost. Med* 1981, 6: 75-90.
- [66] Rao GHR, Johnson GJ, Reddy KR. Ibuprofen Protects Platelet Cyclooxygenase from Irreversible Inhibition by Aspirin. *Arteriosclerosis.* 1983, 3: 383-388.
- [67] Connellan JM, Thurlow PJ, Barlow B. et al. Investigation of alternative mechanisms of collagen-induced platelet activation using monoclonal antibodies to glycoprotein 11b-111a and fibrinogen. *Thromb. Haemost.* 1986, 55: 153-157.
- [68] Muller JE, Tolfer GH. Triggering and hourly variation of onset of arterial thrombosis. *Ann Epidemiol.* 1992, 2: 393-405.
- [69] Rao GHR. Circadian Variations and coronary artery disease. *Chronobiol* 1994, 21: 63-64.
- [70] Prikryl P, Siegelova J, Cornelissen G et al. Chronotherapeutic treatment daily low-dose aspirin. University of Minnesota *Medtronic Chronobiology Series* 1991.
- [71] White JG, Rao GHR, Gerrard JM. Effects of nitroblue tetrazolium and Vitamin E on platelet ultrastructure, aggregation and secretion. *Am J. Pathol* 1978, 92:745-53.
- [72] Rao GHR, Cox CA, Gerrard JM. Et al. Alpha tocopherol quinone: a potent inhibitor of platelet function. *Prog. Lipid Res.* 1981, 6: 51-64.
- [73] Rao GHR, Tate MR, Murthy M. et al. Influence of antioxidants on arachidonic acid metabolism and platelet function. *Biochem Med. and Met Biol.* 1994, 51:74-79.
- [74] Rao GHR, Radha E, White JG. Effect of docosahexaenoic acid (DHA) on arachidonic acid metabolism and platelet function. *Biochem Biophys Res Comm.* 1983, 117: 549-556.
- [75] Pollack A. For Some, Aspirin May Not Help Hearts. *New York Times.* 2004, July.
- [76] Weber AA, Przytuski B, Schanz A. et al. Towards a definition of aspirin resistance: a typological approach. *Platelets.* 2002, 13: 37-40.
- [77] Yilmaz MB, Balbay Y, Korkmaz S. Aspirin resistance. *Anadolu. Kardiyol. Derg* 2004, 4: 59-62.
- [78] Patrono C, Coller B, FitzGerald GA. et al. Platelet-Active Drugs: The Relationships Among Dose, Effectiveness, and Side Effects. *CHEST* 2004, 126: 234S-264S.
- [79] Howard PA. Aspirin resistance. *Ann Pharmacother.* 2002, 36: 1620-1624.
- [80] Hurlen M, Seijeflot I, Arnesen. The Effect of Different Regimens on Platelet Aggregation After Myocardial Infarction. *Scand. Cardiovasc. J.* 1998, 32: 233-237.
- [81] Wu KK, Hoak JC. A new method for the quantitative detection of platelet aggregation in patients with arterial insufficiency. *Lancet* 1974, 11: 924-926.
- [82] Gum PA, Kottke-Marchant K, Poggio ED. et al. Profile and prevalence of aspirin resistance in patients with

- cardiovascular disease. *Am. J. Cardiol.* 2001; 88: 230-235.
- [83] Gum PA, Kottke-Marchant K, Welsh PA. et al. A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease. *J. Am. Coll. Cardiol.* 2003; 41: 961-967.
- [84] Deliagryris E, Boudoulas H. Aspirin Resistance. *Hellenic J. Cardiol.* 2004; 45: 1-5.
- [85] Grottemeyer KH. Effects of acetylsalicylic acid in stroke patients; evidence of non-responders in a subpopulation of treated patients. *Thromb. Res.* 1991; 63: 587-593.
- [86] Grottemeyer KH. Two-year follow-up of aspirin responder and aspirin non-responder. A pilot-study including 180 post-stroke patients. *Thromb. Res.* 1993; 71:397-403.
- [87] Mueller MR, Salat A, Stangi P. et al. Variable platelet response to low-dose ASA and the risk of limb deterioration in patients submitted to peripheral arterial angioplasty. *Thromb. Haemost.* 1997; 78: 1003-1007.
- [88] Eikelboom JW, Hirsh J, Weitz JI. et al. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. *Circ.* 2002; 105: 1650-1655.
- [89] Smout J, Stansby G. Aspirin resistance. *Brit. J. Surgery* 2002; 89: 4-5.
- [90] Helgason CM, Bolin KM, Hoff JA. et al. Development of aspirin resistance in persons with previous ischemic stroke. *Stroke* 1994; 25: 2331-2336.
- [91] Zimmerman N, Wenk A, Kim U. et al. Functional and biochemical evaluation of platelet aspirin resistance after coronary artery bypass surgery. *Circ.* 2003; 108: 542-547.
- [92] Sane DC, McKee SA, Malinin AI. Frequency of aspirin resistance in patients with congestive heart failure treated with antecedent aspirin. *Am. J. Cardiol.* 2002; 90: 893-895.
- [93] Muller I, Besta F, Schulz C. et al. Prevalence of Clopidogrel non-responders among patients with stable angina pectoris scheduled for elective coronary stenting. *Thromb. Haemost.* 2003; 89: 783-787.
- [94] Lau WC, Gubrel PA, Watkins PB. et al. Contribution of hepatic cytochrome P450 3A4 metabolic activity to the phenomenon of Clopidogrel resistance. *Circ.* 2004; 109: 166-171.
- [95] Angiolillo DJ, Fernandez-Ortiz A, Bernardo E. et al. High Clopidogrel loading dose during coronary stenting: effect on drug response and inter-individual variability. *Eur. Heart J.* 2004; 25: 1903-1910.
- [96] Serebruany VL, Steinhubl SR, Berger PB. et al. Variability in platelet responsiveness to Clopidogrel among 544 individuals. *J. Am Coll. Cardiol.* 2005; 45: 246-251.
- [97] Dziewierz A, Dubek D, Heba G. et al. Inter-individual variability in responses to Clopidogrel with cardiac diseases. *Polis Heart J.* 2005; 62: 1-6.
- [98] Altman R, Lucardi HL Muntaner J. et al. The anti-thrombotic profile of aspirin, aspirin resistance or simply failure? *Thromb. J.* 2004; 2: 1-8.
- [99] De Gaetano G, Cerletti C. Aspirin resistance: a revival of platelet aggregation tests? *J. Thromb. Haemost.* 2004; 1: 2048-2061.
- [100] Malinin A, Sperling M, Muhlestein B. et al. Assessing aspirin responsiveness in subjects with multiple risk factors for vascular disease with a rapid platelet function analyzer. *Blood Coag. Fibrinol.* 2004; 1%: 295-301.
- [101] Sambola A, Heras M, Escolar G. et al. The PFA-100 detects sub-optimal antiplatelet responses in patients on aspirin. *Platelets.* 2004; 1-8.
- [102] Coleman JL, Wang JC, Simon DI. Determination of individual responses to Aspirin therapy using the Accumetric Ultegra. *The J. Near-Patient Testing and Technol.* 2004; 3: 77-82.
- [103] Feuring M, Hasseroth K, Janson CP. et al. Inhibition of platelet aggregation after intake of acetylsalicylic acid detected by a platelet function analyzer (PFA-100). *Int. J. Clin Pharmacol Ther.* 1999; 37: 584-548.
- [104] Andersen K, Hurlen M, Arnesen H. et al. Aspirin-responsiveness as measured by a PFA-100 in patients with coronary artery disease. *Thromb. Res.* 2002; 108: 37-42.
- [105] Singh S, Kothari SS, Bahl VK. Aspirin Resistance: Myth or Reality? *Ind. Heart J.* 2003; 55: 1-8
- [106] McKee SA, Sane DC, Deliagryris. Aspirin resistance in cardiovascular disease: A review of prevalence, mechanisms, and clinical significance. *Thromb. Haemost.* 2002; 88: 711-715.
- [107] Eikelboom JW, Hankey GJ. Aspirin resistance: a new independent predictor of vascular events? *J. Am. Coll. Cardiol.* 2003; 41: 966-968.
- [108] Berger PB. Resistance to antiplatelet drugs: Is it real or relevant? *Cath. And Cardiovasc Interven.* 2004; 62: 43-45.
- [109] Shemesh CG, zehavi M, Dinur I et al. Lack of aspirin effect: aspirin resistance or resistance to taking aspirin? *Am. Heart J.* 2004; 147: 293-300.
- [110] Cattaneo M. Aspirin and Clopidogrel: efficacy, safety, and the issue of drug resistance. *Arterioscler. Vasc. Biol.* 2004; 24: 1980-87.
- [111] Nguyen TA, Diodati JG, Pharand C. Resistance Clopidogrel: a review of the evidence. *J. Am Coll Cardiol* 2005; 45: 1157-64.
- [112] Sztritha LK Sas K, Vecsei L. *Aspirin resistance in stroke:* 2004. 2005, 230:163-69.
- [113] Coma-Canella I, Velasco A, Castano S. Prevalence of aspirin resistance measured by PFA-100. *Int. J. Cardiol.* 2005, 101:71-76.

- [114] Kuliczkowski W, Halawa B, Korolko B. Aspirin resistance in ischemic heart disease. *Kardiol Pol.* 2005, 62: 14-25.
- [115] Schwartz KA, Schwartz DE, Ghosheh K et al. Compliance as a critical consideration in patients who appear to be resistant to aspirin after healing of myocardial infarction. *Am. J. Cardiol* 2005, 95: 973-75.
- [116] Driegier A, Dudek D, Heba G, Inter-individual variability in response to Clopidogrel in patients with coronary artery disease. *Kardiol Pol.* 2005, 62: 108-118.
- [117] Serebruany VL, Steinhubl SR, Berger PB. et al. Variability in platelet responsiveness to Clopidogrel among 544 individuals. *J Am Coll Cardiol.* 2005 45: 246-51.
- [118] Rao GHR. Aspirin resistance: A fact or a myth? *Expt. and Clin. Cardiol.* 2005, 10: 17-20.
- [119] Pettersen AA, Seljeflot I, Abdelnoor M et al. Unstable angina, stroke, myocardial infarction and death in aspirin non-responders. A Prospective, randomized trial. The ASCET (Aspirin non-responsiveness and Clopidogrel Endpoint Trial) design. *Scand Cardiovasc. J.* 2004, 38:353-56.



## ARTICLE

# Circadian Cardiology

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## Abstract

A circadian cell cycle resides in every cell, and peripheral timing mechanisms are documented in molecular biologic terms at about 24-hour (circadian) and higher (ultradian) frequencies with coordination in mammals by the adrenal and the pineal-hypothalamic-pituitary network. The suprachiasmatic nuclei (SCN) and clock gene contribute to the coordination of the circadian rhythms' phase and amplitude in everyday life. The SCN are influenced by the daily alternation between light and darkness directly via the eyes and by plasma melatonin concentrations secreted by the pineal gland, which is a window to both light and geomagnetics. A clinical event occurs when the neuroendocrine time structures (chronomes) are not able to cope with the adverse effects of stimuli acting from within or from without, e. g., via the sympathetic nervous system. Triggering of the neuroendocrines by environmental factors may activate the pineal gland, pituitary functions and adrenal secretions, resulting in adverse effects on circadian variations, heart rate variability (HRV) and blood pressure variability (BPV). Circadian rhythm has been known to ancient man from the time of Homoerectus and Homosapiens who used to have intercourse in the early morning hours, prior to going hunting in the forests, causing increased secretion of testosterone in the morning. The role of time-adjusted drug intake, especially in the early morning, was also known to ancient Indian physicians. In the Ayurveda, drinking of large amounts of water in the early morning is advised, which appears to be in an attempt to increase vagal tone due to gastric distention. Frey considered the mean distribution of deaths along the scales of the day and the year. In one industrial population, Pell and D'Allonzo, discussed time-macroskopically the occurrence of a peak in the morning hours in a study of acute myocardial infarction (AMI), a proposition also ascertained and extended to the yearly pattern time-microscopically. The subsequent reports from other countries, the Soviet Union and the extensive data by WHO in the report of myocardial infarction Community Registers from 19 European centers demonstrated a peak incidence of onset of chest pain due to AMI from 8.00 to 11.00 AM with a ratio of approximately 2. In one study from India, in 605 AMI patients, 39% of those who had Q wave infarction (n=174) had the onset between 6. 00 AM to 12. 00 noon. In a more recent Mechanism of Acute Myocardial Infarction (MAMI) study by Singh et al, among 202 AMI patients, the incidence of onset of chest pain was highest

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in the second quarter of the day (41. 0%), mainly between 4-8 AM, followed by the 4<sup>th</sup> quarter, usually after large meals (28. 2%). Emotion was the second most common trigger (43. 5%), which was the most common in the patients with onset of chest pain in the second quarter of the day (51. 8%). Cold weather was a predisposing factor in 29. 2% and hot temperatures (40°C) was common in 24. 7% of the patients. A large meal, especially a large breakfast in the morning was an important trigger of AMI in this study, which poses the intriguing possibility that some heart attacks could be prevented by not eating breakfast or by eating a small superfood breakfast containing walnuts, almonds, raisins and yogurt which are known to be protective against cardiovascular disease. Blood pressure is usually lower during the night, starts increasing before awakening, and remains high during the day-time, mostly in the second quarter of the day. Circadian rhythms of triggers and cardiac events and their mechanisms have been described by various workers. It is possible that circadian rhythm could be a new target for treatment with lifestyle changes, nutrient, nutraceuticals such as w-3 fatty acids and coenzyme Q10, prayer and drug therapy in the management of cardiovascular disease.

## **Introduction**

Many scientists believe that our universe came into existence about 20 billion years ago after an explosion of a primordial fireball of matter. Within 3 minutes of the explosion, the elementary matter particles coalesced to form the lightest elements, which were responsible for the formation of galaxies. Most of the galaxies appear to be receding from us at high velocities, which has been explained by the expansion model of the universe. Rhythm is a fundamental characteristic of the galaxies in our universe. The sun revolves around the core of the Milky Way Galaxy, rotating on its axis. The planets revolve around the sun and moon revolves around the planets. One season follows the other season, day follows night, tide-in follows tide-out. Most of the rhythms are linked to the movements of the heavenly bodies. The rotation of the earth is responsible for the day and night cycle, and the moon orbiting around the earth for the monthly cycle. The procession of the earth on its axis is responsible for the four seasons and the earth orbiting the sun for the annual cycle. Many physiological functions must adapt to these external rhythms, causing dysfunctions resulting in the development of diseases. [1-3].

The universe of living creatures appears to have similar characteristics. All living cells have life cycles and periodicity in their life function [3]. The migration of birds, the hibernation of bears, dogs and several other animals, ripening of fruits and flowering of plants, are driven by the changing seasons. Similarly, other rhythms, such as sleep and wakefulness, the opening and closing of flowers, feeding and nesting are also driven by the circadian cycle. There is substantial evidence that cardiovascular events occur in the second quarter of the day. [1-3]. The exact pathogenesis and risk factors of circadian rhythms are not known [4-9].

## **The Interaction of Genes and Means and Adaptation**

Diet and lifestyle, tobacco, pollutants, stress, and alcohol are the most important environmental means, which interact with genes in the pathogenesis of cardiovascular disease, diabetes and cancer. The interaction of genes and environment, nature and nurture, is the foundation for the development of disease and health. It has been shown through molecular biological techniques that genetic factors determine susceptibility to disease and environmental factors determine which genetically susceptible individual will develop a disease [9]. Despite major changes in our diet and lifestyle in the last 10,000 years, our genes have not changed and hence our biological responses are unable to adapt, resulting in greater adverse effects of these factors on our circadian rhythms. The spontaneous mutation rate for nuclear DNA is estimated at 0.5% per million years. The period of 10,000 years of the agricultural revolution appears to be too short to modulate our genes, causing an approximate 0.005% alteration. It seems our genes are much similar today to our ancestors of the Paleolithic period 40,000 years ago, when our genetic profile was established [9]. It is possible to presume that humans today live in an environment that differs completely from that when our genetic constitution was selected and therefore our clock gene remains as potent as several million years ago to initiate the biorhythms of our bodies. Our clock gene does not allow us to adapt to modulate

rapid changes in diet and lifestyle, which influence circadian rhythms.

## Evolutionary Aspects of Biological Function Rhythmicity

Homosapiens, along with their predecessors *Homo-habilis*, were primarily vegetarians, although for more than a million years, they have been hunting. They became omnivorous as they moved away from other primates. Environmental influences, development of social groups and increasing demands for survival made them skillful and caused significant changes in their lifestyle. They went to the forest in the early morning for hunting animals. They were killed early in life due to accidents. It has been proposed that, in order to preserve their progeny, the Homosapiens had intercourse early in the morning resulting in one of the most important physiological adaptations of an increased release of testosterone in the morning, as a circadian rhythm. The increased release of testosterone occurred to coordinate sexual activity with the aim of conceiving the women. We suggest that since hunting used to start at about sunrise, causing enormous excitement, physical and mental exertion and increased sympathetic activity (associated with marked secretion of cortisol, catecholamines, aldosterone, angiotensin and renin in a state of low melatonin), it also became a circadian rhythm in modern men. Ancient man was able to adapt and fight the adverse effects of testosterone, cortisol and catecholamines, due to their enormous physical activity and lack of time for breakfast. Modern men is not, however, resulting in increased rhythmicity of cardiac events in the second quarter of the day. Late night falling asleep, late awakening, heavy breakfasts, no physical activity and meditation in the morning, and excitement related to rushing to the workplace, trigger a biochemical and biological atmosphere in our body that contributes to the greater cardiovascular events and deaths between 8.00-11.00 hours. [1-3].

We are comparing the diet and lifestyle as well as heart rate and blood pressures around the clock for 7 days in people in tribes living in the adverse conditions of Laddakh, with those living in Moradabad and Tokyo City to determine the pattern

of circadian changes, and to provide further insight into the pathogenesis of circadian rhythms.

## Evolutionary Aspects of Diet and Lifestyle

Recently, several scholars became interested in health and disease from an evolutionary perspective and consider whether our departure from the hunter-gatherer lifestyle may contribute to contemporary health problems [1, 2].

Foods are probably appropriate for any given species if they were regularly consumed during most of the prior evolution, as in the case of hunter gatherers. Prehistoric human hunter gatherers may have protected themselves by choosing plants that have low concentrations of xenobiotics and by avoiding dangerous plant species. The available food staples during the Palaeolithic period may have been meat, fish, shellfish, leafy vegetables, fruits, nuts, insects and larvae in varying proportions. Since tubers often require cooking for their starch to be digestible their routine consumption may have started in the later period. [6-8].

Prior to the agricultural revolution, about 10,000 years ago, humans ate an enormous variety of wild plants. Meat, fish, green leafy vegetables, fruits, nuts, berries and honey were the food items available to pre-agricultural humans which shaped modern humans' genetic nutritional requirements. Early man also managed to survive in a wide range of environments, from the tropical rainforest to near the Arctic, each of these environments offering a different and limited set of foods. However, all diets of ancient men lacked breakfast foods; milk, cheese, butter, syrups, cereals, and of course, refined carbohydrates and sugar that is sprinkled on them, which are known to have adverse effects on our metabolisms causing insulin resistance. These foods supply approximately 70% of the energy in the diets of the Western world today. Irrespective of the specific proportions of meat and vegetables, prehistoric hunter-gatherers' diets would have been very different from those of contemporary humans with regard to their nutritional value.

Hunting was the major part of the occupational physical activity of prehistoric men, which might

have continued throughout the entire day. After they returned from the forest in midday or evening, they may have had a late heavy lunch, followed by dancing and eating again in the late evening. Many experts suggest that reversion to the original Palaeolithic human lifestyle (1.5 million-10,000 years BP) might be useful in the prevention of chronic diseases of Western populations [6-8]. The main symbols of Gods of early men were the sun, fire, sea and hills, which they worshiped to decrease their mental stress.

Several biological functions, such as temperature regulation, blood circulation in the nasal mucosa, the

caliber of the tracheobronchial airway, blood pressure, heart rate, cortisol and growth hormone release exhibit an intrinsic circadian rhythm [1-5]. Most physiological rhythms may be ultradian having a duration of seconds, minutes, or at most a few hours. Circadian means 24 hours, circamensual, 30 days and circaannual, one year. The sleep phases, heart rate, respiratory rate and the functional peaks of many enzymes, receptors and endothelial nitric oxide releases show ultradian variations, depending upon triggers and clocks. (Table 1).

**Table 1. Neuropsychiatric risk factors of acute myocardial infarction**

Precursors	Healthy Subjects (n=595)	AMI (n=202)
Anxiety	88 (14. 8)	122 (60. 4)*
Depression	42 (7. 0)	45 (22. 3)*
Type A behaviour	103 (17. 3)	95 (47. 2)*
Emotional stress	147 (24. 2)	92 (45. 5)*
Sleep deprivation	42 (7. 0)	56 (27. 7)*
Cold Climate	-	59 (29. 2)*
Hot climate (>40 degree celsius)	-	50 (24. 7)*
Large Meals	147 (24. 7)	96 (47. 5)*
Physical Exertion	173 (29. 1)	63 (31. 2)*
Diabetes mellitus	70 (11. 7)	53 (26. 2)*

\*= $P<0.05$  by Chi square test

## Circadian

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 for the first time in 1955 [4, 5]. Chronobiology developed globally after 1969 when an article entitled "Chronobiology" was published in the *Annual Review of Physiology* and became a Current Contents citation classic. Body temperature shows circadian variation; it is lowest in the morning, peaks in the afternoon and falls again during the night. In contrast, cortisol release peaks in the morning. Blood pressure varies by up to 40mm Hg over the time structure of the day with a clear-cut peak in the morning.

Cholesterol is synthesized mainly in the nighttime. An annual variation has also been reported in aldosterone secretion showing a peak during the winter. The cycle of the reproductive organs is the

best-known circamensual rhythm. Ultradian sleep phases (up to a few hours), heart rate, respiratory rate, circadian temperature changes in the interior of the body, all have a circadian rhythm. A circaannual rhythmicity has been observed for serum cholesterol levels with the winter values being much higher. Halberg feels that apart from circadian, circaseptan (weekly), monthly, yearly or even longer term physiological variations occur. Otsuka proposed that the physiological chronomes such as heart rate variability have counterparts in our environment and our genetic makeup over time may have evolved from our adaptation to and integration with the cosmos. There is a need to examine how these phenomena including heliomagnetics and geomagnetics, can influence physiological chronomes, especially for heart rate and blood pressure variability [10].

## Clocks

The circadian rhythms enable the body to adapt in an optimum manner to changes in its environments including geomagnetic forces. These rhythms are set up endogenously in the body and the entire system of hormonised rhythms, the biological time structure can be influenced by external and internal factors, which are called clocks. It is very difficult to assess the internal factors that govern the circadian rhythms. Hence, we know very little about internal clocks.

It is indeed very difficult to assess the internal factors that govern biological rhythms, however. Halberg has proposed that a circadian cycle resides in every living cell and peripheral timing mechanisms are being documented in molecular biological terms at about 24 hour (circadian) and ultradian frequencies, with coordination in mammals by the Adrenal, pineal-hypothalamic-pituitary network (Figure 1). Suprachiasmatic nuclei (SCN) is one known specific area of the brain that works as an internal clock and is responsible for the regulation of central nervous functions, phase and amplitude, as it receives information from the eyes. The information from the

brain is transferred to the pineal gland. Melatonin released from the pituitary gland during the dark hours of the days in a circadian cycle regulates sleep and awake periods. The SCN are influenced by the daily alternation between light and darkness directly via the eyes and melatonin levels. A cardiovascular event occurs when our neuroendocrine time structures (chronomes) are not able to cope with the adverse effects of stimuli from within or without acting eg via the sympathetic nervous system [10-13]. It appears that the two most powerful external clocks for humans are light at high intensity and social contacts. The results of initial attempts to use bright light to adapt shift workers were encouraging [14]. Most organisms from cyanobacteria to mammals are known to use circadian clocks to coordinate their metabolisms with the natural circadian light/dark cycle. The human clock gene was discovered and mapped to chromosome 17p12-13. 1 in 1997 [15]. It is surprising that the clock gene is similar in all life forms on earth. In plants, several molecular components have been described for the circadian system.

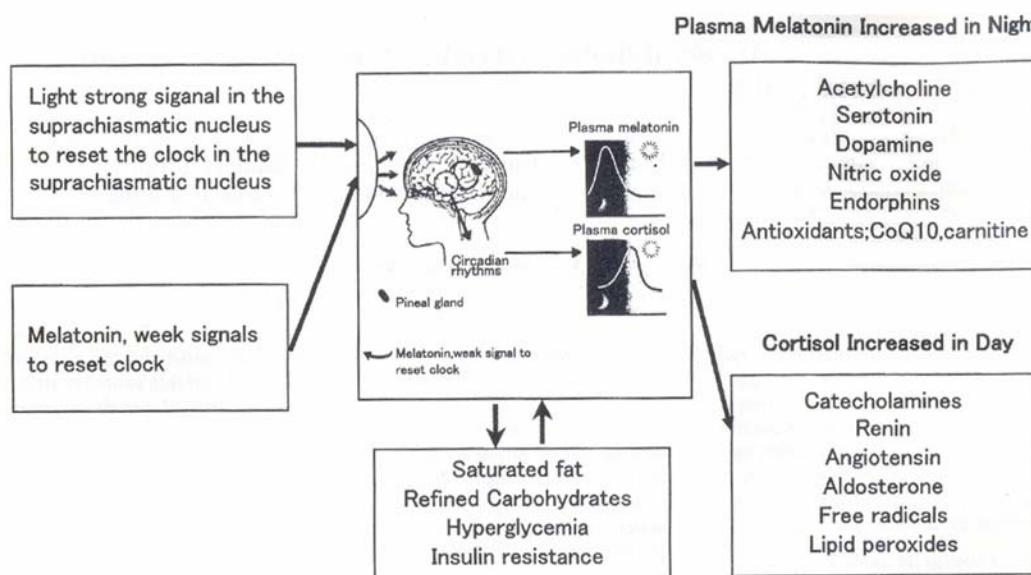
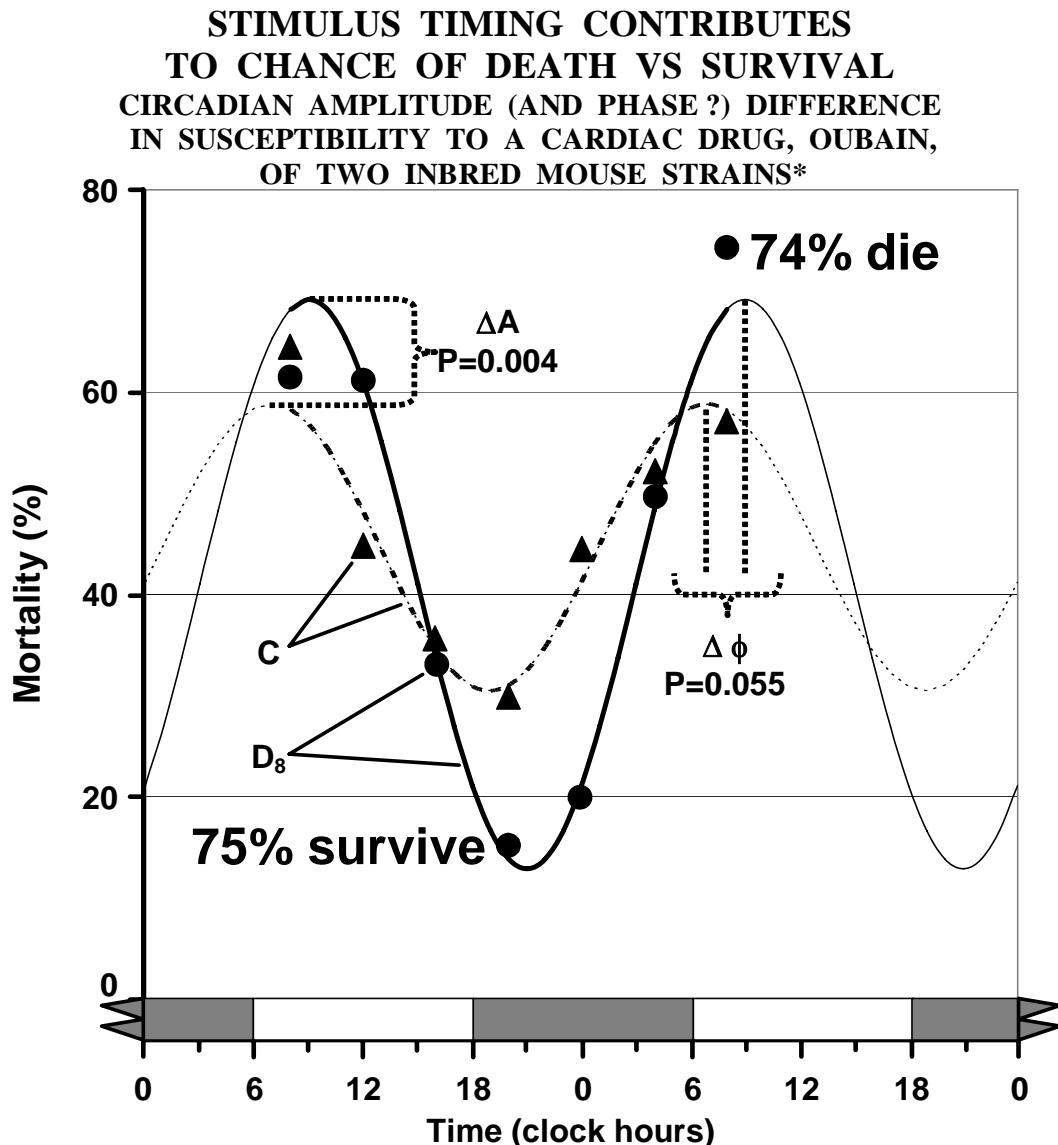


Figure 1. Control of Daily Rhythms By Molecular Clock



Strain	P	M	Amplitude (A)	Acrophase ( $\phi^{**}$ )
D <sub>8</sub>	<b>P &lt; 0.001</b>	<b>40.0</b>	<b>28.1 (21.5; 34.8)</b>	<b>-135 (-119; -150)</b>
C	<b>P = 0.011</b>	<b>45.2</b>	<b>14.2 (7.3; 21.0)</b>	<b>-104 (-73; -136)</b>

\* C = Bagg albino, D<sub>8</sub> = subline 8 of Dilute Brown strain; ouabain 0.15 or 0.5 mg/20 g of body weight, respectively. Thin lines in the graph are extrapolations of fitted cosine curves. \*\* In the table: 95% confidence limits in parentheses,  $\phi$  in degrees (360° = 24 hours). M = MESOR; P from zero-A (no-rhythm) test.

Data from F.Halberg et al., Minnesota Acad Sci Proc 1959; 27: 139-143.

Figure 2. First demonstration of the importance of timing with a drug, in this case > a cardiac drug and also a demonstration more generally of the importance of statistics along the circadian scale. Feel free to use it.

## Triggers

Triggering of the chronomes by environmental factors may activate the pineal gland, pituitary functions and adrenal secretions, causing adverse effects on circadian rhythms, heart rate variability and blood pressure variability [10-14], resulting in cardiovascular events [11-15]. The various clinical manifestations of cardiovascular events do not occur at random times, but according to a time structure. It is possible that certain external activities known as triggers may play a major role in causing myocardial ischaemia, myocardial infarction, sudden cardiac death and stroke [11-15]. The MILIS (Multicentre Investigation of Limitation of Infarct Size) study showed that half of all myocardial infarction patients reported a temporal relationship between characteristic activities and occurrence of infarction. Emotional stress, mild and heavy physical exertion, sleep deprivation and large meals were the most frequently reported triggering activities [16].

The TRIMM (Triggers and Mechanisms of Myocardial Infarction) study showed that 76% of all infarction patients reported an unusual event shortly before the onset of infarction [17]. Emotional upset or stress was reported by 52% of the subjects and 10%

reported multiple trigger activities coinciding just before the event. A morning increase in platelet aggregation appears to be one of the most important triggers for cardiovascular events in the second quarter of the day [18]. It seems that there is powerful evidence of a link between these triggers and the sequences of cellular and pathophysiological events that are proposed to be responsible for coronary artery ischaemia. In one study among 58 patients with AMI, large meals including a large breakfast, emotional stress, cold climate and hot climate ( $>40^{\circ}\text{C}$ ) were important triggers. A large breakfast in the morning was associated with increased concentration of proinflammatory cytokines; tumour necrosis factor-alpha and interleukin-6 as well as insulin and glucose in the blood [11] (Table 2). In the MAMI study [2], large meals, sleep deprivation, emotional stress, cold climate and hot climate were quite commonly observed (Table 3). Circadian rhythms of myocardial ischaemia, angina pectoris, myocardial infarction, sudden cardiac death and stroke have been described by several experts which are triggered by environmental factors as well as biochemical and biological triggers present in every living cell.

**Table 2. Clinical Characteristics of subjects in various subgroups in relation to circadian pattern**

Sub Group	1-6 hours	6-12 hours	12-18 hours	18-24 hours
Total n=202	34 (16. 8)	83 (41. 0)	28 (13. 8)	57 (28. 2)
Male (n=160)	24 (15. 0)	60 (37. 5)	25 (15. 6)	51 (31. 8)
Female (n=132)	10 (23. 8)	23 (54. 7)	3 (7. 1)	6 (14. 2)
Age>60 (n=70)	12 (17. 1)	32 (45. 7)	8 (11. 4)	18 (25. 7)
60 and less (n=132)	12 (16. 6)	51 (38. 6)	20 (15. 1)	39 (29. 5)
History of CAD	16 (30. 7)	24 (46. 1)	7 (13. 4)	5 (9. 6)
Known Diabetes (n=53)	10 (18. 8)	22 (41. 5)	8 (15. 0)	13 (24. 5)
Known Hypertension (n=75) ( $>140/90$ )	13 (17. 3)	30 (40. 0)	11 (14. 6)	21 (28. 0)

Values are number (%). P value was obtained by comparison of subgroups by chi square test. \*P<0. 05m \*=P<0. 02, CAD= Coronary artery disease

## Circadian Rhythms in Ischemic Heart Disease

In the decade since the 1985 observation by Muller et al. that the frequency of onset of myocardial infarction peaks at 9 a. m., numerous publications have supported this observation not only for

myocardial infarction, but also for sudden cardiac death, transient myocardial ischemia, and stroke. Refinement of these epidemiological observations has led to the conclusion that the morning peak in disease onset is due in part to the physical and mental stressors associated with morning awakening and activity. The observations also showed that stressors

such as heavy physical activity and anger can trigger acute cardiovascular events [19].

## **Why Morning Is so Risky?**

Circadian rhythm peaking in the morning is also found for so-called silent myocardial ischaemia, which occurs in more than 20% of patients with arterial hypertension, and can be regularly detected in combined 24-h-ABPM/EKG examinations. Comparative studies have shown that hypertensives with SMI suffer more cardiac events than those with no SMI. It has further been demonstrated that an elevated blood pressure amplitude, which is considered an independent risk factor for cardiac events, is associated with an increased incidence of SMI in patients with micro- or macro-angiopathy [20].

## **The Possible Mechanisms Involved**

Transient myocardial ischemia, detected by ambulatory ST segment monitoring, is also unevenly distributed during the day. The pathophysiology and the mechanisms underlying these variations are the

focus of much investigation. Heart rate, blood pressure, neural and humoral vasoactive factors such as plasma norepinephrine levels and renin activity, and probably also contractility, are increased in the morning hours, indicating that an increase in myocardial oxygen demands contribute importantly to the increased prevalence of ischemia in the morning. A study by Li JJ found that the circadian rhythm of the ischemic threshold detected by repetitive exercise treadmill tests in patients with chronic coronary artery disease is also apparently associated with levels of plasma ET-1 [21].

### *Relation to Plasma Endothelin-1*

Li JJ et al. demonstrated that ET-1 values were higher in the morning hours as compared to afternoon hours. Circadian variations of plasma levels of ET-1 are likely to be one of the most likely mechanisms involved of reduction of the ischemic threshold in the morning hours [22].

**Table 3. Triggers observed in 202 patients of acute myocardial infarction in relation to circadian periods**

Quarter of 24 hrs Subjects	1-6 hours 34	6-12 hours 83	12-18 hours 28	18-24 hours 57	Total 202
Trigger total	25 (73. 5)	70 (84. 3)	15 (53. 5)	42 (73. 7)	162 (82. 2)
Large meals	18 (52. 9)	41 (49. 4)	12 (42. 8)	26 (45. 6)	96 (47. 5)
Emotional stress	15 (44. 1)	43 (51. 8)	14 (50. 0)	20 (35. 0)	92 (45. 5)
Physical Exertion	12 (35. 3)	25 (30. 1)	8 (28. 5)	18 (31. 6)	63 (31. 2)
Sleep deprivation	8 (23. 5)	32 (38. 5)	6 (21. 4)	10 (17. 5)	56 (27. 7)
Cold climate	10 (29. 4)	35 (42. 1)	5 (17. 8)	9 (15. 5)	59 (29. 2)
Hot climate (>40 celsius)	8 (23. 5)	30 (36. 1)	5 (17. 8)	7 (12. 3)	50 (24. 7)

Values are number (%) \*= $P<0.05$ , Pvalues were obtained by comparison of subgroups by chi square test

### *Fluctuation in Endothelial Function*

Coronary spasms are induced by acetylcholine (ACh), which causes vasodilation when the endothelium is functioning normally. The spasms are promptly relieved by nitroglycerin (NTG), which causes vasodilation through its direct action on the

smooth muscle. The frequency of ischemic attacks displays diurnal variations in patients with VA (i. e., coronary spastic angina). The number of attacks increases in the early morning and decreases in the afternoon.

### *Mechanism of Coronary Spasms*

In patients with coronary spasms, coronary dilation caused by endothelium-dependent vasodilators is reported to be impaired. In addition, antioxidants such as vitamin C and glutathione, attenuate the constrictor response to ACh. The plasma levels of vitamin E, a natural antioxidant, are decreased. The coronary arteries of patients with VA exhibit a circadian variation in tone. As the endothelial function of the brachial artery and that of the coronary artery are closely related [22], the fluctuation of endothelium-dependent dilation in the brachial artery most likely takes place in the coronary arteries as well.

### *Mechanism of Diurnal Variation in Flow-Mediated Vasodilation*

It is known that the day/night pattern in the intensity of physical activity causes diurnal variation of hemodynamics. In the evening and afternoon, peripheral blood flow to the skeletal muscle is increased through local regulation in proportion to its need for oxygen and nutrients. Endothelial nitric oxide synthase is known to be upregulated by increased blood flow or shear stress.

Therefore, it is possible that the variation in baseline blood flow may up regulate nitric oxide synthase and contribute to the fluctuation of the flow-mediated, endothelium dependent vasodilation in patients with VA. Diurnal fluctuations of the endothelial function may play an important role in the occurrence of ischemic episodes in patients with VA [23].

### *Heart Rate Variability and the Rate of Autonomic Change*

The incidence of sudden cardiac death peaks during the early morning hours when there is a rapid withdrawal of vagal and an increase of sympathetic tone. The rate of autonomic change could be of prognostic importance. B. Wennerblom showed that during the night/morning hours, healthy controls demonstrated faster HF max. velocity and higher HF

gradient than angina patients. The authors concluded that rapid vagal withdrawal seemed to be a sign of a healthy autonomic nervous system in the control group but was significantly slower in angina patients. IS-5-MN and metoprolol tended to normalise vagal withdrawal and metoprolol slowed down the rapid increase in sympathetic predominance in the morning in these patients [24].

### **Circadian Rhythm in Myocardial Infarction**

Despite impressive strides in diagnosis and management over the past three decades, acute myocardial infarction (AMI) continues to be a major public health problem in the industrialized world and is becoming an increasingly important problem in developing countries. About 50 percent of the deaths associated with AMI occur within 1 hour of the event and are attributable to arrhythmias, most often ventricular fibrillation. Because AMI may strike an individual during the most productive years, it can have profoundly deleterious psychosocial and economic ramifications [25].

### *Circadian Periodicity*

An analysis of a large number of patients hospitalized with MI, studied as a part of the Multicenter Investigation of Limitation of Infarct Size (MILIS), revealed a pronounced circadian periodicity for the time of onset of AMI, with peak incidence of events between 6 a. m. and 12 p. m. About 20% of myocardial infarctions occur between midnight and 6 AM. The circadian distribution of acute myocardial infarction has a morning peak, especially during the first few hours after awakening [26].

### **Pathogenesis**

Circadian rhythms affect many physiological and biochemical parameters; the early morning hours are associated with rises in plasma catecholamines and cortisol as well as increases in platelet aggregability. At this time, sympathetic activation is thought to

disrupt atherosclerotic plaque through vasoconstriction and an increase in blood pressure. In addition, early morning changes in hemostatic activity may lead to thrombosis. In contrast, sleep may be protective, both because of the absence of external triggers and the decline in sympathetic activity during non-rapid eye movement (REM) sleep, which accounts for about 80% of total sleep time. However, about 20% of myocardial infarctions occur between midnight and 6 AM. REM sleep, which is characterized by sympathetic surges, might be involved—as it is in vasospastic angina—but REM sleep constitutes less than 20% of total sleep time in adults [27].

Hence, the pathogenesis of nocturnal myocardial infarctions may differ from those that occur during the daytime, since they may not be related to sympathetic activation or triggering events. Other sleep-induced cardiovascular changes that affect the coronary circulation or cardiac function may be involved. For example, sleep-induced variations of flow patterns in coronary arteries might be involved, or shifts in blood volume might affect the dimensions and hemodynamic function of the right ventricle [28, 29].

## **Circadian Variation in Plaque Rupture**

Pathologic studies have revealed that plaque rupture is one of the major causes of AMI. One large-scale multicenter study has demonstrated that AMI is 1.28 times more likely to begin between 6:00 A. M. and 12:00 P. M. than during the other three 6-hour intervals of the day<sup>2</sup>. Pathologic studies have revealed that plaque rupture and subsequent thrombosis is one of the major causes of AMI. Therefore, the hypothesis that increased physiologic activities in morning hours may trigger plaque disruption has been proposed [30].

A. Tanaqa et al. demonstrated that in the plaque rupture group, a morning increase (from 6:00 A. M. to 12:00 P. M) was observed, whereas in the nonrupture group there was a nocturnal nadir (from 12 to 6:00 A. M) and no significant morning increase was detected. 53% of the patients in the nonrupture group had preinfarction angina and only 31% of patients had an AMI at rest. This circadian pattern was not detectable

in the nonrupture group. This morning increase in the incidence of plaque rupture accounts for the characteristic circadian rhythm of AMI [31]. Various physiologic studies have highlighted the fact that systemic physiologic processes increase in intensity in the morning, such as an arterial pressure surge accompanied by an increase in heart rate [32] and increased vascular tone [33]. Serum cortisol levels also decrease during the period of increased plaque disruption. Because the Japanese population of patients with AMI compared with whites shows a higher incidence of spasm and greater vasoconstriction of nonspastic segments after acetylcholine [34], this decrease in serum cortisol levels could enhance the sensitivity of the coronary arteries to the vasoconstrictive effects of catecholamines. Such physiologic alterations may, alone or in combination, account for the morning increase in plaque rupture. When plaque rupture occurs, the contents of the lipid core that form the most thrombogenic components of the plaque may be released into the lumen and precipitate a cascade that produces thrombosis. Increased platelet activity, increased blood viscosity, and the minimal level of fibrinolytic activity may further contribute to thrombosis in this setting during the morning hours. The efficacy of  $\beta$  blockers and aspirin in preventing AMI indirectly lends support to the idea that these physiological variations may play an etiologic role in plaque disruption and resultant thrombosis [31].

Several studies have shown that in many instances, MI may occur without any obvious precipitating events and may occur at rest. Grines et al [35] reported that one independent predictor of a higher frequency of onset during the morning hours was the absence of a history of angina.

## **Q-Wave Versus Non-Q Wave MI**

V. Culic et al. [36] demonstrated that both AMI types showed a nonuniform daily distribution of onset with a night nadir (0 to 4 A. M.), and Q-wave AMI also showed a late morning peak (8 A. M. to noon). The study demonstrated that wakeful patients with Q-wave infarction were more likely to have AMI onset in the presence of a possible external trigger than those with non-Q-wave AMI. This is consistent with

the hypothesis that powerful hemodynamic stresses may produce more extensive plaque rupture, often leading to complete occlusion of the coronary lumen and Q-wave infarction. Non-Q-wave infarction is more likely to result from a shorter occlusion of a major coronary artery, with earlier and more frequent reopening than from firm occlusion of a small branch.

An acceptable explanation for a diverse circadian pattern in 2 AMI types, with late morning peak only in the Q-wave AMI, is that hemodynamic,

prothrombotic, and vasoconstrictive forces resulting from a morning rise in sympathetic activity and cortisol levels contribute to the formation of sustained coronary occlusion and Q-wave infarction. A lower incidence of both infarction types during the early morning hours corresponds to the time when most patients would be expected to be asleep, so hemodynamic and other triggering forces may be reduced in patients with both types of AMI.

**Table 4. Circadian pattern of acute myocardial infection in relation to drug therapy**

Subgroups	1-6 hours 34	6-12 hours 83	12-18 hours 28	18-24 hours 57	Total 202
Beta-blockers Calcium	10	8	6	6	30
Blockers	2	2	2	6	12
ACE-inhibitor	4	4	-	6	14
Fish Oil Coenzyme	5	2	-	2	9
Q10	8	2	12	4	26
No Drug Therapy	(4. 3)	64(54. 7)**	10(8. 5)	37(31. 6)**	117
Drug Therapy Total	29(34. 1)	19(22. 3)	18(21. 2)	20(23. 5)	85

Values are number (%) out of totals = P<0. 05, \*\*P=P<0. 01m O value were obtained by comparison of sub-groups by Chi square test

**Table 5: Clinical characteristics of subjects**

	Acute myocardial infarction (n=54)	Controls (n=85)
Sex- males	45 (83. 3)	76(89. 4)
Mean age (years)	49. 5 $\pm$ 4. 2	52. 1 $\pm$ 5. 2
Body mass index (kg/m <sup>2</sup> )	23. 7 $\pm$ 3. 2	22. 4 $\pm$ 3. 4
Hypertension (>140/90 mmhg)	25(46. 3)*	25(29. 4)
Glucose intolerance	12(22. 2)*	10(11. 8)
Diabetes mellitus	14(25. 9)	--
Smoking	24(44. 4)*	28(32. 9)
Higher transfattyacids (>5g/day)	27(50. 0)**	--
Large meals(>1000 Kcal)	27(50. 0)**	25(29. 4)
Large breakfast(>1000 Kcal)	22(40. 7)**	10(11. 8)

\*=P <0. 05 P value was obtained by z score test for proportions by comparison of two groups. Values are number (%) and mean(Standard deviation)

**Table 6: Circadian rhythm of cardiac events in patients with acute myocardial infarction**

Quarters of the day	Number of subjects (%)
1-6 Hours	10(18. 5)
6-12 Hours	20(40. 7)**
12-18 Hours	7(12. 9)
18-24 Hours	15(27. 7)*

=P <0. 05, \*\*=P>0. 02 , P value was obtained by Z score test for proportions

**Table 7. Laboratory data in patients of acute myocardial infarction at baseline and after 4 weeks in relation to meal size**

Data	Large breakfast, (n=22)		Small breakfast, (n=32)	
	Baseline	After4	Baseline	After4
Lipoprotein(a)mg/dl	23.1+5.4	20.1+4.2*	22.5+4.6	19.7+4.1*
Triglycerides (mmol/L)	1.88+0.61	1.70+0.38*	1.81+0.60	1.64+0.32*
Blood glucose(mmol/l)	7.7+1.6	6.0+1.2*	6.6+1.4*	5.5+0.30*
Plasma insulin(mg/dl)	47.5+11.3	36.3+5.6**	43.2+8.8*	27.6+3.5*
TBARS(pmol/l)	1.87+0.46	1.32+0.33*	1.77+0.42	1.30+0.31*
MDA(pmol/l)	2.68+0.34	2.02+0.21*	2.66+0.33	2.01+0.21*
Diene conjugate(OD)	27.5+4.2	24.6+4.0*	26.2+4.1	24.2+3.5*
CoenzymeQ10(ug/ml)	0.21+0.02	0.32+0.23*	0.23+0.03	0.45+0.24*
Interleukin-6(pg/ml)	32.6+6.2	22.5+4.3*	27.5+5.2*	20.6+0.22*
TNF-alpha(ug/dl)	42.5+12.8	23.6+4.1*	38.2+10.6*	19.6+0.18*
	32.6+6.2	22.5+4.3*	27.5+5.2*	20.6+0.22*
	42.5+12.8	23.6+4.1*	38.2+10.6*	19.6+0.18*

\* = P < 0.05, \*\*=P<0.002, TNF=Tumour necrosis factor, MDA=malonaldehyde

**Table 8: Circadian distribution of cases in relation to 4 hourly period.**

Periods	Men	Women	Total
0-4 hours	6(3.7)	2(4.7)	8(3.9)
4-8 hours	58(36.2)*	22(51.7)*	80(39.6)*
8-12 hours	25(15.6)	4(9.4)	29(14.3)
12-16 hours	8(5.0)	-----	8(3.9)
16-20 hours	40(25.2)*	10(23.5)*	50(24.7)*
20-24 hours	23(14.3)	4(9.4)	27(13.3)

Values are numbers (%) \*= $P<0.05$  P values are obtained by comparison of subjects in various sub groups of periods.

**Table 9: Biochemical data in patient with acute myocardial infarction compared to healthy subject.**

Data	Healthy subjects (n=595)	Acute myocardial infarction (n=202)
Serum magnesium (m EQ/L)	1.64(0.25)	1.51(0.23)*
Serum potassium (m EQ/L)	4.3(0.96)	3.7(0.82)*
Vitamin C (mmol/l)	37.5(6.8)	18.6(4.3)*
Vitamin E(mmol/l)	20.1(3.7)	14.5(2.5)*
Vitamin A(mmol/l)	2.25(0.19)	1.86(0.15)*
Interleukin-6 (pg/ml)	15.6(3.2)	30.5(5.7)*
Tumour necrosis factor-alpha (pg/dl)	18.8(4.5)	41.6(8.4)*
Insulin (mg/dl)	17.6(4.2)	48.8(12.8)*

Results of a study by V. Culic suggest that pain at different body areas and other relevant symptoms more frequently follow Q-wave infarction. Both sympathetic and vagal afferent fibers can transmit cardiac pain to remote body areas due to convergence

of visceral and somatic fibers on the same neurons within the central nervous system. Accordingly, a greater area of myocardial damage and more extensive stimulation of afferent fibers could induce pain sensation in a greater number of somatic regions

and cause a higher frequency of other symptoms in patients with Q-wave AMI. The study also demonstrated that dyspnea was more associated with non-Q-wave infarction. A similar frequency of pulmonary congestion in 2 AMI type subsets, even in the presence of lower left ventricular ejection fraction among patients with Q-wave infarction, has been attributed to acute transient myocardial ischemia associated with non-Q-wave infarction, whereas irreversible ischemia could be the dominant mechanism of ventricular dysfunction in patients with Q-wave AMI [37].

## Circadian Differences in the Site of Myocardial Infarction and in the Involved Coronary Arteries

P. Moruzzi and colleagues [27] demonstrated that 55% of the infarctions were anterior or anterolateral and 45% were inferior, including posterior or involving the right ventricle. They found that there was a slight peak in the incidence of myocardial infarction between 6 AM and noon. Anterior infarctions were more common from 6 AM to midnight, whereas inferior infarctions were more common between midnight and 6 AM.

Coronary involvement varied by the time of symptom onset among patients with inferior infarctions: right coronary artery involvement was much more common than left circumflex artery involvement among those with symptom onset from midnight to 6 AM. Among those with right coronary artery involvement, the proximal segment was more common as the site of the culprit lesion between midnight and 6 AM than between 6 AM and midnight.

The right coronary artery, which maintains its large caliber down to the crux, is characterized by slow flow velocity [38, 39]. It is possible that by decreasing the pressure gradient across a coronary artery stenosis, sleep-induced reduction in aortic pressure increases distal stasis, thereby facilitating thrombosis. This may be more of a problem in the right coronary artery than in the left coronary artery because the pressure gradient is inversely related to the fourth power of the minimum lumen diameter [27].

If the rhythmic processes that drive the circadian rhythm of myocardial-infarction onset can be identified their modification may delay or prevent the occurrence of infarction.

## Circadian Rhythm and Sudden Cardiac Death

Sudden cardiac death (SCD) is natural death due to cardiac causes, heralded by abrupt loss of consciousness within 1 hour of the onset of acute symptoms in a person with or without known preexisting heart disease, but in whom the time and mode of death are unexpected. The worldwide incidence of SCD is difficult to estimate but estimates for the United States range from 300, 000 to nearly 400, 000 SCDs annually [40].

## Circadian Rhythm and SCD

The biologic functions of mammals vary according to a circadian rhythm of about 24 hours, which is governed by the hypothalamus. These rhythms can be altered by disease and the actions of drugs can be affected by the time of day at which they are administered. A number of important physiological parameters such as heart rate, blood pressure, vascular reactivity, cardiac contractility, and various hemostatic factors all demonstrate a circadian pattern similar to that described for cardiovascular disorders. Circadian patterns have been observed for several supraventricular arrhythmias, premature ventricular beats, ventricular tachycardias including sudden cardiac death [41].

Sudden cardiac death has a circadian pattern, with the peak incidence occurring from 7 to 11 A. M. [42], from 7 to 9 A. M., with more sudden cardiac deaths occurring between 6 A. M. and 12 P. M. than during the other quarters of the day [43]: from 9 A. M. to 12 P. M. [44], and from 6 A. M. to 12 P. M. [45]. A study by Aronow and colleagues [46] showed that there was a circadian pattern in the number of deaths per hour in patients with CHF after prior myocardial infarction. The primary peak occurred between 6 A. M. and 12 P. M., with 39% of deaths occurring in that 6-hour period.

Flack and Yunis [47] indicate that the morning peak from 6 am to 12 noon of such occurrences as MI, thrombotic stroke, transient myocardial ischemia, and SCD is due to the high levels of coronary vasomotor tone, plasma catecholamines, and platelet aggregability, and the low levels of coronary blood flow and plasma fibrinolytic activity. At this same time, there is a rapid increase in blood pressure during the early morning hours. They also feel that it is important to maintain control of blood pressure throughout the early morning to try to avoid adverse events. A study in Auckland, New Zealand, by Van der Palen et al [48] had some slightly different findings on circadian rhythm. They report an afternoon peak in SCD (32. 5%) and a secondary morning peak (27. 6%). Also, they found a Saturday high (18. 6%) for SCD. Peckova and colleagues [49] showed a similar pattern of cardiac arrest: a low incidence at night, a sharp increase between 8 AM and noon with peaking at 10 AM, a relatively high incidence during the day, and another peak between 5 and 8 PM. Both morning and evening peaks were of a similar magnitude. Young patients had a relatively higher incidence at night than older patients and also exhibited a higher incidence in the morning than in the evening. Patients in the age category 41 to 65 years had a higher evening peak. Elderly patients maintained a rather high incidence during the daytime hours, with less pronounced peaks.

## Mechanisms Underlying Sudden Cardiac Death

Cardiac arrest may have either a cardiac or a non-cardiac aetiology and may present with a variety of arrhythmias, including ventricular tachycardia, ventricular fibrillation, asystole, and electromechanical dissociation. The likelihood of a “sudden death” episode occurring and being witnessed is not constant throughout the day. Soo and colleagues [50] observed that there were three major time periods when a cardiac arrest was most likely to occur—an *early* peak in the morning around 1100 hours, a *late* peak in the evening around 2200 hours, and, for those whose arrest was from a cardiac disease, a third time period—around 1600 hours—also seemed to be critical In Seattle [51] and Berlin

[52], the most important times for a cardiac arrest seemed to be around 1000 and 1800 hours. Ventricular fibrillation was most frequent around 2000 hours in the series by Peckova and colleagues[49], while Arntz and associates found that ventricular fibrillation occurred most often around 1000 hours, with the exception of ventricular fibrillation, the circadian variation of the arrest rhythm in both cardiac and non-cardiac groups appeared to be identical. The triggers for a cardiac arrest in both groups are likely to be similar when the arrhythmia is something other than ventricular fibrillation, irrespective of the aetiology of arrest [51].

## Electrical Factors and Sudden Cardiac Death

The mechanisms underlying sudden cardiac death are probably multifactorial, and in some patients, primarily electrical factors or an interaction between electrical abnormalities and myocardial ischemia may be responsible for sudden cardiac death. Ambulatory recordings at the moment of sudden death have shown that ventricular tachycardia degenerating into ventricular fibrillation is a common terminal rhythm and often occurs in the absence of antecedent ischemic ST segment shifts [52]. In addition, the frequency of complex ventricular ectopy and the occurrence of sustained monomorphic ventricular tachycardia also are greatest in the early morning hours when the incidence of sudden death is greatest. Thus, a circadian variation in cardiac electrophysiological parameters may be an alternate mechanism contributing to the circadian variation in sudden death [53].

The distribution of ventricular fibrillation events followed the general pattern except that the evening peak is substantially higher than the morning peak. Peckova and colleagues [49] suggest the evening peak to be of approximately the same magnitude as the morning peak of incidence. They found that the bimodal pattern is mainly an attribute of ventricular fibrillation and that patients with asystole or pulseless electrical activity had a less pronounced evening peak. There was no difference in circadian variation between sexes, races, or days of the week. The analysis suggested that differences between the age categories are probably explicable by different rhythm

distributions and thus are more likely due to sociodemographic factors than to biological differences.

### *Circadian Variation and Refractoriness*

The circadian variation in the incidence of sudden cardiac death mainly appears to be due to variability in the time of onset of ventricular fibrillation [53]. Evidence has provided confirmation of the traditional hypothesis that ventricular fibrillation is due to multiple functional reentrant circuits, which change over time [54, 55]. Shorter refractory periods promote reentry by allowing reentrant circuits to be maintained in a smaller mass of tissue and by decreasing the length of lines of the block, leading to the variability in reentry observed during ventricular fibrillation. Experimental studies also have demonstrated that shortening of refractory periods under a variety of conditions may be proarrhythmic [56]. In the present study, maximal shortening between hourly refractory periods as well as the shortest absolute refractory periods were observed around the hour of waking, raising the possibility of a close relation between these findings and the increased morning incidence of arrhythmic sudden death. Although not measured in this study, an increased dispersion of refractoriness, which has been shown to promote ventricular arrhythmias [57], might be expected when overall ventricular refractoriness is rapidly changing. The circadian variation in refractory periods may be associated with a circadian variation in other electrophysiological properties, such as conduction velocity, which also may promote the emergence of reentrant circuits and arrhythmias. Fluctuations in sympathetic tone are mainly responsible for temporal changes in ventricular refractoriness [53].

Studies also have shown that acute cardiac events are temporally linked to waking [58]. In the Physicians' Health Study [59], 25% of myocardial infarctions occurred within 3 hours of waking, and the relative risk of infarction during this time interval was almost twice that during any other 3-hour period during the day. In the study by Kong QT et al [53], the most pronounced changes in ventricular refractoriness were also more closely linked to the hour of waking than to the absolute time of day. Results of the study

therefore suggest that the time around waking is characterized by marked electrophysiological changes and possibly an increased vulnerability to arrhythmias.

### *Circadian Variation and Repolarization*

The QT interval as measured on a surface ECG reflects global ventricular myocardial repolarization. A circadian variation in the QT interval has been described previously [61] and has been attributed to a circadian variation in ventricular repolarization. The QT interval has been found to be longest during sleep and shortest during the waking hours. However, the relevance of these observations to a potential circadian variation in ventricular refractoriness is not completely clear because the refractory period and action potential duration (as reflected globally by the QT interval) may be dissociated in some situations [62]. In addition, evaluation of the QT interval at different times of the day requires a correction for heart rate, which could limit the accuracy of the analysis of circadian changes in the QT interval. Finally, unlike refractory periods that were generally reproducible to within 4 ms with the methodology used in the study by Kong QT and colleagues [53], estimation of the QT interval may be somewhat less reproducible. Despite these potential differences, the results of the study suggest that the circadian variation in ventricular refractoriness qualitatively parallels the previously described circadian variation in the QT interval.

Yi et al [62] examined circadian variation of the QT interval in post MI patients and normal controls and its relation to SCD. They found the SCD victims did not show a significantly longer QT interval at night when compared to day as the normal subjects and MI survivors did, and the SCD victims did show a significantly longer QTc averaged over 24 hours. So, the circadian variation of the QT interval did vary when compared to normal subjects and those who survived the year following a MI.

### **Circadian Variation in Serum Potassium and Plasma Catecholamine Levels**

Kong QT and colleagues [53] demonstrated a significant circadian variation in mean serum potassium levels, with the highest levels observed during the waking hours and the lowest levels during sleep. In eight of nine subjects, the minimum potassium levels were observed between the hours of 1:00 AM and 4:00 AM; the ninth subject had a minimum level at 2:00 PM.

A significant circadian variation was also observed in mean levels of plasma epinephrine and norepinephrine. Mean levels of each were highest during the day and lowest during sleep. A single harmonic model with a peak at 3:00 PM provided the best fit for epinephrine levels, and a double harmonic model with peaks at 9:00 AM and 9:00 PM provided the best fit for norepinephrine levels. We also observed low levels of magnesium and potassium as well as vitamine E and C and higher levels of TBARS and MDA, which are indicators of oxidative damage, in the second quarter of the day compared to evening values.

### **Circadian Variation in Cardiac Events**

Some of the circadian variability in the incidence of sudden death may be due to a morning increase in myocardial ischemia [63, 64, 65, 66]. However, ischemic events do not account for all instances of sudden cardiac death, as autopsy studies of sudden death victims have failed to demonstrate acute coronary lesions in up to 42% of cases [67]. Furthermore, the occurrence of sustained monomorphic ventricular tachycardia, which is not generally a consequence of acute ischemia [68], also demonstrates a substantial variation [69]. Therefore, a circadian variation in cardiac electrophysiological parameters could potentially contribute to the increased morning incidence of sudden cardiac death.

### **Circadian Rhythm and Stroke**

#### *Monday Preference in Onset of Ischemic Stroke*

The onset of stroke has a specific temporal pattern [70], characterized by a higher frequency in winter and in the mornings. According to a meta-analysis of more than 11, 000 patients, an estimated 37% of strokes occurred during morning hours [71].

A circaseptan rhythm, with a significant peak on Mondays was observed for all strokes and for ischemic strokes.

Data from a Norwegian study [72] suggested that biochemical factors associated with cardiovascular risk—such as measures of hematosis and carbohydrate and lipid metabolism—were less favorable on Mondays compared with other days of the week. Thus, similar to the association between increased thrombophilia in the mornings [73] and the circadian pattern of other thrombotic disease such as myocardial infarction [74] and limb ischemia [75], the Monday risk of ischemic stroke may reflect an increased thrombogenic condition [76].

#### *Circadian Variation in Acute Ischemic Stroke*

The existence of a circadian rhythm in ischemic stroke has been established, with a higher frequency in the early hours of the morning. The cause of this diurnal variation is uncertain. It has been related to the circadian rhythm of fibrinolysis, platelet aggregability [77] and mainly, arterial blood pressure, with its minimum value during sleep and maximum value in the early hours in the morning, in both normotensive and hypertensive patients [78]. However, it has been suggested that an increase in morning stroke onset could be due to patients awakening with neurological deficits as a result of a stroke that could have occurred during the night [79].

A. Lago et al. [79] found a higher frequency of stroke during the day and a lower frequency between 6:01 PM and 12:00 AM, obvious in all the different types of ischemic stroke viz. lacunar, thrombotic, embolic stroke.

In acute myocardial ischemia, the time of onset is easily determined [80, 81] corresponding with the

onset of thoracic pain. This does not occur when the patient with ischemic stroke is discovered upon awakening; the onset may have occurred at any time during sleep. Unfortunately, at present there is no marker that indicates the time of stroke onset.

Stroke is classified within a heterogeneous group of vascular diseases, unlike myocardial ischemia, with different etiopathogenic mechanisms. Arterial pressure, as other factors, may play an important role, favoring an increase in morning stroke onset. But these same factors, such as lower arterial pressure and heart rate during the night may contribute, through a hemodynamic mechanism, to a stroke onset during the sleeping hours, particularly in the case of thrombotic stroke. Results of study by A. Lago et al. [79] show that thrombotic and lacunar strokes have a higher onset during sleeping hours when compared with embolic stroke. The circadian rhythm of arterial blood pressure may be disturbed in patients who have suffered from stroke.

### *Loss of Circadian Rhythm of Blood Pressure Following Acute Stroke*

Hypertension remains a dominant risk factor and prognostic indicator in patients with stroke in all communities. The risk of stroke is directly related to elevations of blood pressure.

S. Jain et al [82] showed a pathologically reduced or abolished circadian BP variation after stroke. The absence of normal dipping results in a higher 24 hour blood pressure load and may have more target organ damage than those with normal diurnal variations of blood pressure. The epidemiology of acute stroke is different in developing countries than that in the developed world. The age at stroke, risk factors, subtypes of stroke and prognosis are different in developing countries. In India, ischemic strokes constitute 70–75% while hemorrhagic strokes account for 20-25% of total cases [82]. However, hypertension remains a dominant risk factor and prognostic indicator in patients with stroke in all communities. The risk of stroke is directly related to elevations of blood pressure.

The accurate measurement of blood pressure after an acute stroke is important because antihypertensive therapy may be considered in some patients.

However, blood pressure may be falsely elevated or depressed immediately after a stroke depending on the level of consciousness, severity of neurological deficit and physical activity.

The normal diurnal variation in blood pressure i.e., nighttime dipping was abolished in 88% patients in study by Jain and colleagues [82]. This nondipping was seen equally in both hemorrhagic and ischemic subgroups without any statistically significant difference.

In studies of patients with stroke, abnormal pattern of circadian rhythm of blood pressure using ambulatory blood pressure monitoring (ABPM) has been reported [83-85]. In a different study, Dawson et al[86] found a significant reduction in diurnal variation in systolic blood pressure in cortical infarct and intracerebral hemorrhage subgroups, compared with control subjects. The subcortical infarct subgroup demonstrated only minimal reduction in normal circadian variation. Fujishima et al [87] reported that blood pressure was elevated in the acute phase of a single lacunar infarction and it declined with time. No night time fall was noted in acute phase, but the circadian variation in blood pressure normalized in the subacute and chronic phase.

### *Circadian Variation in the Timing of Stroke Onset*

Early studies of the timing of acute stroke, however, indicated that many afflicted patients reported awakening with new neurologic deficits, and several reports indicated that acute strokes tended to occur either during the evening hours or during sleep [88]. This led to the conclusion that especially because acute therapies for stroke-in-evolution were not particularly effective, there was little reason to consider acute stroke as a medical emergency because the onset of symptoms was thought to occur during sleep, when most patients would not recognize them [89].

The highest risk is found between 8:01 AM and noon; the lowest is found between midnight and 4 AM. Because there are some reports from Japan [90], especially regarding hemorrhagic stroke [91], which suggest that there may be differences in circadian variation of stroke timing according to the subtype of

stroke of interest, meta-analyses of ischemic and hemorrhagic stroke (including subarachnoid and intracerebral bleeds), and transient ischemic attack were also carried out. Results of the study by WJ Elliott and colleagues [89] suggest that for each subtype of stroke studied, there is an increase in risk during the early morning hours. The data are remarkably consistent across the various subtypes of stroke, and indicate, for ischemic stroke, hemorrhagic stroke, and even transient ischemic attacks, that the excess risk during the 6 AM to noon time period is significantly higher than would be expected by chance: 89%, 52%, and 80%. Similarly, there was a significantly lower risk of stroke during the nighttime hours (midnight to 6 AM) for each stroke subtype: 30%, 54%, and 81%.

The finding that the early morning hours (and not the nighttime hours) have the highest risk for the onset of stroke symptoms has two broad implications. The first is that patients should no longer be told that stroke symptoms are not a medical emergency. Although this may have been sound public policy when acute treatments for stroke were not available, there is now some evidence that acute emergent treatments for cerebral ischemia can be delivered in a timely fashion and result in improved long-term outcomes. The results of meta-analysis contradict older conclusions that "strokes are more likely to occur during sleep". Meta-analysis indicates that irrespective of the type of stroke, most patients will be awake when the onset of stroke symptoms occurs. The recognition of new neurologic deficits should prompt afflicted patients and their families to consider these as a medical emergency (or "brain attack"). The second implication has to do with some modalities useful in stroke prevention. Blood pressure is often considered one of the most powerful risk factors for stroke and has a circadian variation that essentially parallels the circadian variation in stroke onset. Antihypertensive agents administered in the morning ought to have a long duration of action to still have an effect on the early morning rise in blood pressure. It is tempting to speculate that antihypertensive agents that specifically target the early morning rise in blood pressure and heart rate, without reducing blood pressure severely during the night, might be more advantageous in controlling the 20% rise in blood pressure during the hours around awakening. This

appears also to be the time of day associated with an increased risk of stroke, myocardial infarction, and sudden cardiac death [89].

## **Circadian and Seasonal Occurrence of Subarachnoid and Intracerebral Hemorrhage**

Subarachnoid hemorrhage (SAH) and intracerebral hemorrhage (ICH) are associated with high morbidity and mortality, and they represent about 20% of all strokes [92].

The occurrence of cerebral infarction has been reported to vary with the time of day; cerebrovascular ischemic events predominantly occur in the early morning and late afternoon [88, 93-95]. Recently, seasonal variations in the incidence of this disorder have also been detected, the peak occurrence being in the winter and autumn [96].

In some studies, the occurrence of SAH in individuals with a history of hypertension has been reported to vary with time of day [97-99].

P.A. Nyquist et al. [96] reported that the occurrence of ICH and SAH combined was increased during the winter. However, when the hemorrhage subtypes were evaluated, the main trend for seasonal differences was noted in SAH but not in ICH.

The circadian variation of blood pressure seems to mirror the time for onset of hemorrhagic stroke in that daily increases in blood pressure occur in the morning and early afternoon and lower levels occur at night [88, 98, 100]. Some investigators have hypothesized that this increase in blood pressure may be responsible for the increase in hemorrhagic stroke frequency in the early morning and afternoon. In addition, others have noted that normotensive individuals who experience SAH or ICH may have a late-afternoon locus of onset instead of an early morning locus of onset [98]. Other authors have suggested that the occurrence of SAH and ICH during the working hours may suggest an activity precipitant for SAH or ICH [101]. Factors that might contribute to an increased risk during the winter are uncertain. However, cardiovascular stresses occur during the winter, particularly in areas with extremes of seasonal climates, which may result in fluctuations in blood pressure, blood coagulability, and cardiovascular

performance. These stresses include deconditioning, stress of physical activity in temperature extremes, types of activity required in a cold climate, and more specific effects of abrupt temperature changes and temperature extremes on the blood pressure and vascular system. Inflammatory factors such as cytokine levels and cortisol levels fluctuate with a circadian and circannual rhythmicity. These factors may play a role in priming the vasculature to respond in a manner predisposing the endothelium to rupture [96].

## Circadian Variation in Transient Ischaemic Attack

A metaanalysis of 31 publications reporting the circadian timing of 11,816 strokes found a 49% increase in stroke of all types between 0600 and 1200 [89]. Possible explanations for the circadian pattern of cerebrovascular events have focused on circadian or postural changes in platelet aggregation, thrombolysis, blood pressure, heart rate, and catecholamine concentrations that occur after awakening with resumption of physical and mental activities. In a minority of cases, which varies in the literature from less than 10% to as much as 44%, stroke occurs at night [88, 93, 102]. This suggests that sleep, although "protective" for most cerebrovascular events, may represent a vulnerable state for a subset of patients at risk for stroke. Nocturnal blood pressure swings, cardiac arrhythmias, and sleep disordered breathing have been suggested as possible explanations for the nocturnal onset of stroke. Not only acute ischaemic stroke but also transient ischaemic attacks have a circadian pattern with a peak of onset between 0600 and 1200 and about 20% of events occurring at night time [103-105]. Furthermore, it is shown for the first time that patients with daytime and night time onset of transient ischaemic attack/stroke are similar with regard to most cardiovascular risk factors; clinical and polysomnographic sleep characteristics; and stroke parameters.

Findings by C. Bassetti et al. [106] suggest that low diastolic blood pressure values may predispose to nighttime onset of cerebrovascular events enhancing the sleep related fall in blood pressure.

## Onset of Transient Ischaemic Attack/Stroke and Sleep Characteristics

Sleep disordered breathing is present in about 50% of patients with acute cerebrovascular diseases [107, 108]. Sleep apnoea—while being a risk factor for stroke [109, 110]—may only rarely represent the immediate cause of transient ischaemic attack or stroke. Rather, respiratory events during sleep may cause haemodynamic and haematological changes which increase the risk of cerebrovascular events at the transition from sleep to wakefulness and during the subsequent few hours.

It is nevertheless conceivable that in a patient with severe sleep apnoea swings in blood pressure, decreased cerebral blood flow, and cardiac arrhythmias produced by respiratory events may trigger cardiovascular and cerebrovascular events during sleep [106].

## Onset of Transient Ischaemic Attack/Stroke and Stroke Characteristics

In a study of 1,233 patients, Lago et al [79] also found a higher frequency of all types of stroke during the day and a higher night time onset in macroangiopathy and microangiopathy compared with other stroke subtypes. Macroangiopathic and microangiopathic strokes may be particularly susceptible to nocturnal haemodynamic changes and decreased cerebral perfusion. Although yet to be proved, it is possible that intrathoracic pressure variations related to sleep apnoea may also predispose to cardioembolic strokes secondary to right-left shunt (for example, patent foramen ovale).

C. Bassetti et al. [106] have thus proven that [107] night time and daytime transient ischaemic attack/ stroke are similar in sleep and stroke characteristics, and [70, 71] diastolic hypotension may predispose to night time cerebrovascular events.

## Nonpharmacological Treatment

Nonpharmacological management of circadian rhythm with yoga, physical activity, moderate alcohol intake, functional foods such as almonds, walnuts, rape seed oil, and neutraceuticals, eg coenzyme Q10 and w-3 fatty acids appear to be protective against circadian rhythms of cardiovascular events.

## Pharmacotherapy

The concept of homeostasis in biology postulates that there is constancy of the internal milieu. Thus, it is assumed the risk and exacerbation of disease are invariable and independent of the time of day, day of month, and month of year as are the responses of patients to diagnostic tests and medications. Findings from the field of chronobiology, the study of biological rhythms, challenge the concept of homeostasis and the many assumptions and procedures of clinical medicine based on it. It is now recognized that human functions have daily, weekly, monthly and yearly biological rhythms. Plants, animals, and insects also have chronobiological rhythms. Circadian patterns have been observed for a variety of cardiovascular disorders, including cardiac arrhythmias, sudden cardiac death, cerebrovascular events, episodes of stable angina, unstable angina and acute myocardial infarction. The morning predominance of these events has been well documented in a number of large population studies. It is now recognized that circadian and other rhythms of the gastrointestinal tract and vital organs are capable of significantly affecting the pharmacokinetics and dynamics of cardiovascular and other medications. This means that the effects of therapeutic interventions administered in identical doses in the morning versus the evening may not be equivalent. The prevention and treatment of cardiovascular disease must take into account chronobiological factors [111].

## Chronotherapeutics

Chronotherapeutics is the delivery of drugs at levels that match the body's needs at certain times of

day or night. Chronotherapy links the effects of a disease to time, and thus the timing of drug delivery. The main objective of chronotherapy for heart disease would be to deliver needed drugs in higher concentrations during the time they are needed the most (early morning after-waking period) ; and at reduced drug levels when the need is less (during the middle of the sleep cycle) [112].

The goal of chronotherapeutics is to match the timing of treatment with the intrinsic timing of illness. Theoretically, optimum therapy is more likely to result when the right amount of drug is delivered to the correct target organ at the most appropriate time. In contrast, many side effects can be minimized if a drug is not given when it is not needed [113].

## Time-Tailored Drugs

Many drugs display normal, reproducible daily variations in pharmacokinetics and pharmacodynamics. Lemmer identified more than 100 drugs that display significant variation in concentrations or effects, or both, over 24 hours. Perhaps the best example is heparin. Even when it is administered at a constant infusion rate, the activated partial thromboplastin time and the risk of bleeding vary significantly according to the hour of the day and are higher at night [114]. The narrower the therapeutic window (i.e., risk-benefit ratio) for a specific drug, the more important the implication of the circadian variation in plasma levels [110].

### *Selected Medications Exhibiting Circadian Variations in Pharmacokinetic and Pharmacodynamic Action*

#### *Cardiovascular agents*

- Enalapril
- Diltiazem HCl
- Nifedipine
- Propranolol HCl
- Verapamil

### ***Antiasthmatic agents***

- Terbutaline sulfate
- Theophylline
- Gastrointestinal agents:
- Cimetidine
- Omeprazole

### ***Nonsteroidal anti-inflammatory drugs***

- Acetylsalicylic acid
- Indomethacin
- Ketoprofen

### ***Anticancer agents***

- Cisplatin
- Doxorubicin
- Methotrexate

### ***Other***

- Diazepam
- Haloperidol

In selecting the most appropriate treatment for diseases that can be managed with chronotherapy, clinicians should understand that pharmacodynamic profiles of chronotherapeutic formulations are often different from those of traditional homeostatic formulations, even when the active drug itself is the same. Such differences may have important clinical consequences. Unlike homeostatic formulations, which provide relatively constant plasma drug levels over 24 hours, chronotherapeutic formulations may use various release mechanisms (e.g., time-delay coatings, osmotic pump mechanisms, matrix systems) that provide for varying levels throughout the day [115].

## **Potential Role of Chronotherapy in Cardiovascular Diseases**

### ***Angiotensin Converting Enzyme Inhibitors***

In one study, an ACE inhibitor was dosed either in the early morning or at bedtime in 18 patients with moderately high blood pressure. Palatini et al showed that nighttime dosing resulted in a greater effect on nighttime pressure than morning dosing. There was no difference in daytime pressure between the 2 groups. Measurement of ACE activity showed that nighttime dosing caused a more even, lasting reduction in blood levels of ACE.

In contrast to the ACE inhibitor study, studies with the beta-blocker atenolol, and the calcium channel blockers nifedipine and amlodipine, showed no different effects on pressure when dosed in the morning versus evening. Like many such studies, too few patients were studied to draw strong conclusions.

It is obvious, though, that drug class, formulation, and size of dose have a large influence on the effect seen [116].

### ***Calcium Channel Blockers***

In a study by Yosefy and colleagues from Israel it was found that long acting calcium channel blockers prevent the rise in blood pressure in morning hours and decreased the incidence of stroke by around 50%; thus providing a comprehensive cover during the dangerous hours in the morning [117].

Currently, preparations of verapamil hydrochloride are recommended for dosing at night to provide higher drug levels in the morning, when blood pressure, heart rate, and the risk of cardiovascular events are highest. Studies in hypertension [118] and angina pectoris [119] have demonstrated the benefits of a chronotherapeutic approach to these conditions.

### ***Aspirin***

The Physicians' Health Study, a randomized, double-blind, placebo-controlled trial of alternate-day

aspirin intake (325 mg) among 22, 071 US male physicians, afforded the opportunity to assess this circadian pattern and examine whether it is altered by aspirin therapy. During a 5-year period of follow-up, 342 cases of nonfatal myocardial infarction were confirmed, of which the time of onset was available in 211 (62%). In the aspirin group, circadian variation was minimal, due primarily to a marked reduction in the morning peak of infarction. Specifically, aspirin was associated with a 59. 3% reduction in the incidence of infarction during the morning waking hours, compared with a 34. 1% reduction for the remaining hours of the day. The greater reduction was observed during the 3-hour interval immediately after awakening, a period with a risk of infarction twice that of any other comparable time interval. Aspirin intake was associated with a mean reduction in the incidence of infarction of 44. 8% over the entire 24-hour cycle. Aspirin reduces the risk of infarction by inhibiting platelet aggregation during the critical periods [118].

### Beta Blockers

Drugs may suppress diurnal variation, specifically b-blockers [120, 121], antiarrhythmic drugs, and aspirin. The fact that b-blockers suppress circadian variation of myocardial infarction was already noted in several studies. If b-blockers also suppress circadian variation of cardiac arrests, we would expect that the interval between the times of day of the two arrests would widen, because the expected difference between the arrest times if no circadian variation is present is larger than in the presence of circadian variation [121].

### Summary

The recognition of circadian rhythms in both normal human biologic function and disease has heightened awareness that the timing of drug regimens may have an important impact on effectiveness of treatment. Outcomes in several diseases that have predictable circadian variations have been improved by matching the timing of medication use to the circadian rhythm of the illness.

### References

- [1] Singh, RB;Weydahl, K;Otsuka K et al. Can nutrition influence circadian rhythm and heart rate variability? *Biomed Pharmacother* 2001, 55:(Supple)115-24.
- [2] Singh, RB;Kartik, C;Otsuka K;Pella D;Pella J. Brain-heart connection and the risk of heart attack. (Editorial). *Biomed Pharmacother* 2002, 56:(Supple)257-265
- [3] Otsuka, K;Cornelissen, G;Halberg, F. Circadian rhythms and clinical chronobiology. *Biomed Pharmacother* 2001, 55:(Supple)7-18.
- [4] Halberg, F;Stephens, AN. Susceptibility to quabain and physiologic circadian periodicity. *Proc Minn Acad Sci* 1959;27:139-43.
- [5] Halberg F;Visscher MB. Regular diurnal physiological variation in eosinophil levels in five stocks of mice. *Proc Soc Exp Biol (NY)*1950, 75:846-47.
- [6] Downing D. The history of man in four diets, editorial. *J Nutr Environ Med* 2003, 13:139-41.
- [7] Trowell HC, Burkitt DP(eds). *Western Disease:their emergence and prevention*. London, Edward Arnold, 1981.
- [8] Eaton SB, Strassman BI, Nesse RM et al. Evolutionary health promotion. *Prev Med* 2002, 34:109-18.
- [9] Simopoulos AP. Genetic variation and dietary response:Nutrigenetics/nutrigenomics. *Asia Pac J Clin Nutr* 2002, 11(S6):117-128.
- [10] Otsuka K, Shohgo M, Kozo M, Zi-yan Z, Weydahl A, Hansen TL. et al. Chronomes, aging and disease. In Furukawa H, Nishibuchi M, Kono Y, Kaida Y editors;*Ecological Destruction, Health and Development:Advancing Asian Paradigms*, 2004, p 367-94.
- [11] Singh RB, Pella D, Rastogi S, Sharma JP, K Kartikey, Goyal VK et al. Increased concentrations of lipoprotein(a), circadian rhythms, and metabolic reactants evoked by acute myocardial infarction in relation to large breakfast. *Biomed Pharmacother* 2004, 58(Supplement):116-122.
- [12] Singh RB, Pella D, Neki NS, Chandel JP, Gupta P, Rastogi S et al. Mechanism of acute myocardial infarction(MAMI) study. *Biomed Pharmacother*, 2004, 58(Supplement):111-115.
- [13] Singh RB, Cornelissen G, Weydahl A, Schwartzkopft O, Katinas G, Otsuka K et al, Circadian heart rate and blood pressure variability considered for research and patient care. *Int J Cardiol* 2003, 87:9-28.
- [14] Czeisler CA, Johnson MP, Duffy JF, Brown EN, Rondo JM, Kronauer RE. Exposure to bright light and darkness to treat physiologic maladaption to night work. *N Engl J Med* 1990, 322:1253-59.
- [15] Tei H, Okamura H, Shigeyoshi Y, Fukuhara C , Ozawa R, Hirose M, Sakaki Y. Circadian oscillation of a mammalian homologue of the *Drosophila* period gene. *Nature* 1997, 389, pp512-516.

- [16] Tofler GH, Stone PH, Maclure M, Edelman E, Davis VG, Robertson T, Antman EM, Muller JE. Analysis of possible triggers of myocardial infarction(The MILIS Study). *Am J Cardiol* 1990; 66:22-27.
- [17] Willich SN, Loewel H, Lewis M, Arntz R, Baur R, Winther K, Keil U, Shroeder R, TRIMM study group. Association of wake time and the onset of infarction. *Z Kardiol* 1991; 80(suppl 3):105-10.
- [18] Tofler GH, Brezenzki D, Schafer AI, Czeisler CA, Rutherford JD, Willich SN, Gleason RE, Williams GH, Muller JE. Concurrent morning increase in platelet aggregability and the risk of myocardial infarction and sudden cardiac death. *N Engl J Med* 1987; 316:1514-18.
- [19] Kozak M. Circadian rhythms in cardiovascular diseases, ischemic heart disease . *Vnitr Lek*. 2003 Apr;49(4):302-7.
- [20] Un S, Baulmann J, Weisser B, Dusing R, Vetter H, Mengden T. Circadian rhythm of silent myocardial ischemia. Why morning is so risky for hypertensive patients. *MMW Fortschr Med.* 2003 Nov 20;145(47):34-8.
- [21] Li JJ. Circadian variation in myocardial ischemia:the possible mechanisms involving in this phenomenon. *Med Hypotheses*. 2003 Aug;61(2):240-3.
- [22] Li JJ; Huang CX; Fang CH; Chen F; Jiang H; Tang QZ; Li GS. Circadian variation in ischemic threshold in patients with stable angina: relation to plasma endothelin-1. *Angiology* - 01-Jul-2002; 53(4): 409-13.
- [23] Kawano H; Motoyama T; Yasue H; Hirai N; Waly HM; Kugiyama K; Ogawa H. Endothelial function fluctuates with diurnal variation in the frequency of ischemic episodes in patients with variant angina. *J Am Coll Cardiol* - 17-Jul-2002; 40(2): 266-70
- [24] Wennerblom B; Lurje I; Karlsson T; Tygesen H; Vahisalo R; Hjalmarson A. Circadian variation of heart rate variability and the rate of autonomic change in the morning hours in healthy subjects and angina patients. *Int J Cardiol* - 01-Jun-2001; 79(1): 61-9.
- [25] Antman ME, Braunwald E. Acute myocardial infarction. In Braunwald (Eds. ): *Heart disease: A Textbook of Cardiovascular Medicine 6<sup>th</sup> ed*; Philadelphia, W. B. Saunders Company, 2001, p. 1114.
- [26] MILIS Study Group. Muller JE, Stone PH, Turi ZG, Rutherford JD, Czeisler CA, Parker C, Poole WK, Passamani E, Roberts R, Robertson T. Circadian variation in the frequency of onset of acute myocardial infarction. *N Engl J Med* 1985;313:1315-22.
- [27] Moruzzi P, Marenzi G, Callegari S, Contini M. Circadian distribution of acute myocardial infarction by anatomic location and coronary artery involvement. *Am J Med.* 2004 Jan 1;116(1):24-7.
- [28] Kirby DA, Verrier RL. Differential effects of sleep stage on coronary hemodynamic function. *Am J Physiol* 1989;256:H1378-83.
- [29] Verrier RL, Dickerson LW. Autonomic nervous system and coronary blood flow changes related to emotional activation and sleep. *Circulation* 1991;83 (suppl II):II-81-9.
- [30] Muller JE, Tofler GH, Stone PH. Circadian variation and triggers of onset of acute cardiovascular disease. *Circulation* 1989;79:733-43.
- [31] Tanaka A, Kawarabayashi T, Fukuda D, Nishibori Y, Sakamoto T, Nishida Y, Shimada K, Yoshikawa J. Circadian variation of plaque rupture in acute myocardial infarction. *Am J Cardiol.* 2004 Jan 1;93(1):1-5.
- [32] Millar-Craig MW, Bishop CN, Raftery EB. Circadian variation of blood-pressure. *Lancet* 1978;1:795-7.
- [33] Fujita M, Franklin D. Diurnal changes in coronary blood flow in conscious dogs. *Circulation* 1987;76:488-91
- [34] Pristipino C, Beltrame JF, Finocchiaro ML, Hattori R, Fujita M, Mongiardo R, Cianflone D, Sanna T, Sasayama S, Maseri A. Major racial differences in coronary constrictor response between Japanese and Caucasians with recent myocardial infarction. *Circulation* 2000;101:1102-8.
- [35] Grines CL, Booth DC, Nissen SE, Gurley JC, Bennett KA, O'Connor WN, DeMaria AN. Mechanism of acute myocardial infarction in patients with prior coronary artery bypass grafting and therapeutic implications. *Am J Cardiol* 1990;65:1292-6.
- [36] Culic V, Miric D, Eterovic D. Different circumstances, timing, and symptom presentation at onset of Q-wave versus non-Q-wave acute myocardial infarction. *Am J Cardiol.* 2002 Feb 15;89(4):456-60.
- [37] Benhorin J., Moss A. J., Oakes D. , Marcus F. , Greenberg H. , Dwyer Jr E. M., Algeo S. , Hahn E. Multicenter Diltiazem Post-Infarction Group. The prognostic significance of first myocardial infarction type (Q wave versus non-Q wave) and Q wave location. *J Am Coll Cardiol* 1990;15:1201-7.
- [38] Ofili EO, Labovitz AJ, Kern MJ. Coronary flow velocity dynamics in normal and diseased arteries. *Am J Cardiol* 1993;71:3D-9D.
- [39] Anderson HV, Stokes MJ, Leon M, Abu-Halawa SA, Stuart Y, Kirkeeide RL. Coronary artery flow velocity is related to lumen area and regional left ventricular mass. *Circulation* 2000;102:48-54.
- [40] Myerburg JR, Castellanos A. Cardiac arrest and sudden cardiac death. In Braunwald (Eds.): *Heart disease: A Textbook of Cardiovascular Medicine 4<sup>th</sup> ed*; Philadelphia, W. B. Saunders Company, 2001, p. 756.
- [41] Kozak M. Circadian rhythms in cardiovascular diseases—arrhythmias. *Vnitr Lek*. 2003 Apr;49(4):297-301
- [42] Muller JE; Ludmer PL; Willich SN; Tofler GH; Aylmer G; Klangos I; Stone PH. Circadian variation in the frequency of sudden cardiac death. *Circulation* - 01-Jan-1987; 75(1): 131-8

- [43] Willlich SN; Levy D; Rocco MB; Tofler GH; Stone PH; Muller JE. Circadian variation in the incidence of sudden cardiac death in the Framingham Heart Study population. *Am J Cardiol* - 1-Oct-1987; 60(10): 801-6.
- [44] Willlich SN; Goldberg RJ; Maclure M; Perriello L; Muller JE. Increased onset of sudden cardiac death in the first three hours after awakening. *Am J Cardiol* - 1-Jul-1992; 70(1): 65-8
- [45] Levine RL; Pepe PE; Fromm RE Jr; Curka PA; Clark PA. Prospective evidence of a circadian rhythm for out-of-hospital cardiac arrests. *JAMA* - 3-Jun-1992; 267(21):2935-7
- [46] Aronow WS, Ahn C. Circadian variation of death from congestive heart failure after prior myocardial infarction in patients >60 years of age. *Am J Cardiol*. 2003 Dec 1;92 (11):1354-5.
- [47] Flack JM, Yunis C. Therapeutic implications of the epidemiology and timing of myocardial infarction and other cardiovascular diseases. *J Hum Hypertens*. 1997 Jan;11(1):23-8.
- [48] Van der Palen J, Doggen CJ, Beaglehole R. Variation in the time and day of onset of myocardial infarction and sudden death. *N Z Med J*. 1995 Aug 25;108 (1006):332-4.
- [49] Peckova M; Fahrenbruch CE; Cobb LA; Hallstrom AP. Circadian Variations in the Occurrence of Cardiac Arrests: Initial and Repeat Episodes. *Circulation*. 1998;98:31-39.
- [50] Soo L H, Gray D, Young T, Hampton JR. Circadian variation in witnessed out of hospital cardiac arrest. *Heart* 2000;84:370-376.
- [51] Arntz HR, Willich SN, Oeff M, Bruggemann T, Stern R, Heinzmann A, Matenaer B, Schroder R. Circadian variation of sudden cardiac death reflects age-related variability in ventricular fibrillation. *Circulation*. 1993 Nov;88(5 Pt 1):2284-9.
- [52] Leclercq JF, Maisonblanche P, Cauchemez B, Coumel P. Respective role of sympathetic tone and of cardiac pauses in the genesis of 62 cases of ventricular fibrillation recorded during Holter monitoring. *Eur Heart J*. 1988 Dec;9 (12):1276-83.
- [53] Thomas Q, Kong, Jr, Jeffrey J. Goldberger, Michele Parker, RN, Ted Wang, Alan H. Kadish. Circadian Variation in Human Ventricular Refractoriness. *Circulation*. 1995;92:1507-1516.
- [54] Cha YM, Birgersdotter-Green U, Wolf PL, Peters BB, Chen PS. The mechanism of termination of reentrant activity in ventricular fibrillation. *Circ Res*. 1994 Mar;74(3):495-506.
- [55] Frazier DW, Wolf PD, Wharton JM, Tang AS, Smith WM, Ideker RE. Stimulus-induced critical point. Mechanism for electrical initiation of reentry in normal canine myocardium. *J Clin Invest*. 1989 Mar;83(3):1039-52.
- [56] Sheridan DJ, Culling W. Electrophysiological effects of alpha-adrenoceptor stimulation in normal and ischemic myocardium. *J Cardiovasc Pharmacol*. 1985;7 Suppl 5:S55-60.
- [57] Kuo CS, Atarashi H, Reddy CP, Surawicz B. Dispersion of ventricular repolarization and arrhythmia: study of two consecutive ventricular premature complexes. *Circulation*. 1985 Aug;72(2):370-6.
- [58] Goldberg RJ, Brady P, Muller JE, Chen ZY, de Groot M, Zonneveld P, Dalen JE. Time of onset of symptoms of acute myocardial infarction. *Am J Cardiol*. 1990 Jul 15;66(2):140-4.
- [59] Ridker PM, Manson JE, Buring JE, Muller JE, Hennekens CH. Circadian variation of acute myocardial infarction and the effect of low-dose aspirin in a randomized trial of physicians. *Circulation*. 1990 Sep;82(3):897-902.
- [60] Bexton RS, Vallin HO, Camm AJ. Diurnal variation of the QT interval--influence of the autonomic nervous system. *Br Heart J*. 1986 Mar;55(3):253-8.
- [61] Rozanski GJ, Jalife J, Moe GK. Determinants of postrepolarization refractoriness in depressed mammalian ventricular muscle. *Circ Res*. 1984 Oct;55(4):486-96.
- [62] Yi G, Guo XH, Reardon M, Gallagher MM, Hnatkova K, Camm AJ, Malik M. Circadian variation of the QT interval in patients with sudden cardiac death after myocardial infarction. *Am J Cardiol*. 1998 Apr 15;81(8):950-6.
- [63] Tofler GH, Brezinski D, Schafer AI, Czeisler CA, Rutherford JD, Willich SN, Gleason RE, Williams GH, Muller JE. Concurrent morning increase in platelet aggregability and the risk of myocardial infarction and sudden cardiac death. *N Engl J Med*. 1987 Jun 11;316(24):1514-8.
- [64] Muller JE, Stone PH, Turi ZG, Rutherford JD, Czeisler CA, Parker C, Poole WK, Passamani E, Roberts R, Robertson T, et al. Circadian variation in the frequency of onset of acute myocardial infarction. *N Engl J Med*. 1985 Nov 21;313(21):1315-22.
- [65] Willich SN, Linderer T, Wegscheider K, Leizorovicz A, Alamerchy I, Schroder R. Increased morning incidence of myocardial infarction in the ISAM Study: absence with prior beta-adrenergic blockade. ISAM Study Group. *Circulation*. 1989 Oct;80(4):853-8.
- [66] Fujita M, Franklin D. Diurnal changes in coronary blood flow in conscious dogs. *Circulation*. 1987 Aug;76(2):488-91.
- [67] Roberts WC. Qualitative and quantitative comparison of amounts of narrowing by atherosclerotic plaques in the major epicardial coronary arteries at necropsy in sudden coronary death, transmural acute myocardial infarction, transmural healed myocardial infarction and unstable angina pectoris. *Am J Cardiol*. 1989 Aug 1;64(5):324-8.
- [68] Davies MJ, Bland JM, Hangartner JR, Angelini A, Thomas AC. Factors influencing the presence or absence of acute coronary artery thrombi in sudden ischaemic death. *Eur Heart J*. 1989 Mar;10(3):203-8.

- [69] Lampert R, Rosenfeld L, Batsford W, Lee F, McPherson C. Circadian variation of sustained ventricular tachycardia in patients with coronary artery disease and implantable cardioverter-defibrillators. *Circulation*. 1994 Jul;90(1):241-7.
- [70] Manfredini R, Gallerani M, Portaluppi F, Salmi R, Fersini C. Chronobiological patterns of onset of acute cerebrovascular diseases. *Thromb Res*. 1997 Dec 15;88(6):451-63.
- [71] Elliott WJ. Circadian variation in the timing of stroke onset: a meta-analysis. *Stroke*. 1998 May;29(5):992-6.
- [72] Urdal P, Anderssen SA, Holme I, Hjermann I, Mundal HH, Haaland A, Torjesen P. Monday and non-Monday concentrations of lifestyle-related blood components in the Oslo Diet and Exercise Study. *J Intern Med*. 1998 Dec;244(6):507-13.
- [73] Brezinski DA, Tofler GH, Muller JE, Pohjola-Sintonen S, Willich SN, Schafer AI, Czeisler CA, Williams GH. Morning increase in platelet aggregability. Association with assumption of the upright posture. *Circulation*. 1988 Jul;78(1):35-40.
- [74] Cohen MC, Rohtla KM, Lavery CE, Muller JE, Mittleman MA. Meta-analysis of the morning excess of acute myocardial infarction and sudden cardiac death. *Am J Cardiol*. 1997 Jun 1;79(11):1512-6.
- [75] Manfredini R, Gallerani M, Portaluppi F, Salmi R, Zamboni P, Fersini C. Circadian variation in the onset of acute critical limb ischemia. *Thromb Res*. 1998 Nov 15;92(4):163-9.
- [76] Manfredini R, Casetta I, Paolino E, la Cecilia O, Boari B, Fallica E, Granieri E. Monday preference in onset of ischemic stroke. *Am J Med*. 2001 Oct 1;111(5):401-3.
- [77] Kelly-Hayes M, Wolf PA, Kase CS, Brand FN, McGuirk JM, D'Agostino RB. Temporal patterns of stroke onset. The Framingham Study. *Stroke*. 1995 Aug;26(8):1343-7.
- [78] Millar-Craig MW, Bishop CN, Raftery EB. Circadian variation of blood-pressure. *Lancet*. 1978 Apr 15;1(8068):795-7.
- [79] Lago A, Geffner D, Tembl J, Landete L, Valero C, Baquero M. Circadian variation in acute ischemic stroke: a hospital-based study. *Stroke*. 1998 Sep;29(9):1873-5.
- [80] Quyyumi AA. Circadian rhythms in cardiovascular disease. *Am Heart J*. 1990 Sep;120(3):726-33.
- [81] Muller JE, Tofler GH, Stone PH. Circadian variation and triggers of onset of acute cardiovascular disease. *Circulation*. 1989 Apr;79(4):733-43.
- [82] Jain S, Namboodri KK, Kumari S, Prabhakar S. Loss of circadian rhythm of blood pressure following acute stroke. *BMC Neurol*. 2004 Jan 6;4(1):1.
- [83] Collins R, MacMahon S. Blood pressure, antihypertensive drug treatment and the risks of stroke and of coronary heart disease. *Br Med Bull*. 1994 Apr;50(2):272-98.
- [84] Sander D, Klingelhofer J. Changes of circadian blood pressure patterns after hemodynamic and thromboembolic brain infarction. *Stroke*. 1994 Sep;25(9):1730-7.
- [85] Yamamoto Y, Akiguchi I, Oiwa K, Satoi H, Kimura J. Diminished nocturnal blood pressure decline and lesion site in cerebrovascular disease. *Stroke*. 1995 May;26(5):829-33.
- [86] Dawson SL, Evans SN, Manktelow BN, Fotherby MD, Robinson TG, Potter JF. Diurnal blood pressure change varies with stroke subtype in the acute phase. *Stroke*. 1998 Aug;29(8):1519-24.
- [87] Fujishima S, Abe I, Okada Y, Saku Y, Sadoshima S, Fujishima M. Serial changes in blood pressure and neurohormone levels after the onset of lacunar stroke. *Angiology*. 1996 Jun;47(6):579-87.
- [88] Marshall J. Diurnal variation in occurrence of strokes. *Stroke*. 1977 Mar-Apr;8(2):230-1.
- [89] Elliott WJ. Circadian variation in the timing of stroke onset: a meta-analysis. *Stroke*. 1998 May;29(5):992-6.
- [90] Hayashi S, Toyoshima H, Tanabe N, Miyanishi K. Daily peaks in the incidence of sudden cardiac death and fatal stroke in Niigata Prefecture. *Jpn Circ J*. 1996 Apr;60(4):193-200.
- [91] Vermeer SE, Rinkel GJ, Algra A. Circadian fluctuations in onset of subarachnoid hemorrhage. New data on aneurysmal and perimesencephalic hemorrhage and a systematic review. *Stroke*. 1997 Apr;28(4):805-8.
- [92] Brown RD, Whisnant JP, Sicks JD, O'Fallon WM, Wiebers DO. Stroke incidence, prevalence, and survival: secular trends in Rochester, Minnesota, through 1989. *Stroke*. 1996 Mar;27(3):373-80.
- [93] Van der Windt C, Van Gijn J. Cerebral infarction does not occur typically at night. *J Neurol Neurosurg Psychiatry*. 1988 Jan;51(1): 109-11.
- [94] Wolf PA, Caplan LR, Foulkes MA. Morning increase in onset of ischemic stroke. *Stroke*. 1989 Apr;20(4):473-6.
- [95] Argentino C, Toni D, Rasura M, Violi F, Sacchetti ML, Allegretta A, Balsano F, Fieschi C. Circadian variation in the frequency of ischemic stroke. *Stroke*. 1990 Mar;21(3):387-9.
- [96] Nyquist PA, Brown RD Jr, Wiebers DO, Crowson CS, O'Fallon WM. Circadian and seasonal occurrence of subarachnoid and intracerebral hemorrhage. *Neurology*. 2001 Jan 23;56(2):190-3.
- [97] Sloan MA, Price TR, Foulkes MA, Marler JR, Mohr JP, Hier DB, Wolf PA, Caplan LR. Circadian rhythmicity of stroke onset. Intracerebral and subarachnoid hemorrhage. *Stroke*. 1992 Oct;23(10):1420-6.
- [98] Kleinpeter G, Schatzer R, Bock F. Is blood pressure really a trigger for the circadian rhythm of subarachnoid hemorrhage? *Stroke*. 1995 Oct;26(10):1805-10.
- [99] Gallerani M, Manfredini R, Ricci L, Cocurullo A, Goldoni C, Bigoni M, Fersini C. Chronobiological aspects of acute cerebrovascular diseases. *Acta Neurol Scand*. 1993 Jun;87(6):482-7.

- [100] Rautaharju PM, Manolio TA, Furberg CD, Siscovick D, Newman AB, Borhani NO, Gardin JM. Ischemic episodes in 24-h ambulatory electrocardiograms of elderly persons: the Cardiovascular Health Study. *Int J Cardiol.* 1995 Sep;51(2):165-75.
- [101] Vermeer SE, Rinkel GJ, Algra A. Circadian fluctuations in onset of subarachnoid hemorrhage. New data on aneurysmal and perimesencephalic hemorrhage and a systematic review. *Stroke.* 1997 Apr;28(4):805-8.
- [102] Chamorro A, Vila N, Ascaso C, Elices E, Schoneville W, Blanc R. Blood pressure and functional recovery in acute ischemic stroke. *Stroke.* 1998 Sep;29(9):1850-3.
- [103] Marsh EE 3rd, Biller J, Adams HP Jr, Marler JR, Hulbert JR, Love BB, Gordon DL. Circadian variation in onset of acute ischemic stroke. *Arch Neurol.* 1990 Nov;47(11):1178-80.
- [104] Wroe SJ, Sandercock P, Bamford J, Dennis M, Slattery J, Warlow C. Diurnal variation in incidence of stroke: Oxfordshire community stroke project. *BMJ.* 1992 Jan 18;304(6820):155-7.
- [105] Haapaniemi H, Hillbom M, Juvela S. Weekend and holiday increase in the onset of ischemic stroke in young women. *Stroke.* 1996 Jun;27(6):1023-7.
- [106] Bassetti C, Aldrich M. Night time versus daytime transient ischaemic attack and ischaemic stroke: a prospective study of 110 patients. *J Neurol Neurosurg Psychiatry.* 1999 Oct;67(4):463-7.
- [107] Bassetti C, Aldrich MS, Chervin RD, Quint D. Sleep apnea in patients with transient ischemic attack and stroke: a prospective study of 59 patients. *Neurology.* 1996 Nov;47(5):1167-73.
- [108] Dyken ME, Somers VK, Yamada T, Ren ZY, Zimmerman MB. Investigating the relationship between stroke and obstructive sleep apnea. *Stroke.* 1996 Mar;27(3):401-7.
- [109] Palomaki H, Partinen M, Erkinjuntti T, Kaste M. Snoring, sleep apnea syndrome, and stroke. *Neurology.* 1992 Jul;42(7 Suppl 6):75-81; discussion 82.
- [110] Partinen M. Ischaemic stroke, snoring and obstructive sleep apnoea. *J Sleep Res.* 1995 Jun;4(S1):156-159.
- [111] Kozák M. Use of chronobiology findings in therapy of cardiovascular diseases. *Vnitr Lek.* 2003 Apr;49(4): 308-13.
- [112] Sica DA, White W. Chronotherapeutics and Its Role in the Treatment of Hypertension and Cardiovascular Disease. *J Clin Hypertens* 2000; 2(4):279-286.
- [113] Elliott WJ. Timing treatment to the rhythm of disease: a short course in chronotherapeutics. *Postgrad Med* 2001;110(2):119-29.
- [114] Reinberg AE. Concepts of circadian chronopharmacology. *Ann N Y Acad Sci* 1991;618:102-15.
- [115] Bhalla A, Singh R, Sachdev A, D'Cruz S, Duseja A. Circadian pattern in cerebro vascular disorders. *Neurol India* 2002;50:526-7.
- [116] White WB, Black HR, Weber MA, et al. Comparison of effects of controlled onset extended release verapamil at bedtime and nifedipine gastrointestinal therapeutic system on arising on early morning blood pressure, heart rate, and the heart rate-blood pressure product. *Am J Cardiol* 1998;81:421-31.
- [117] Frishman WH, Glasser S, Stone P, et al. Comparison of controlled-onset extended-release verapamil with amlodipine and amlodipine plus atenolol on exercise performance and ambulatory ischemia in patients with chronic stable angina pectoris. *Am J Cardiol* 1999;83:507-14.
- [118] Ridker PM, Manson JE, Buring JE, Muller JE and Hennekens CH. Circadian variation of acute myocardial infarction and the effect of low-dose aspirin in a randomized trial of physicians. *Circulation.* 1990 Sep;82(3):897-902.
- [119] Willich SN, Linderer T, Wegscheider K, Leizorovicz A, Alamerchy I and Schroder R. Increased morning incidence of myocardial infarction in the ISAM Study: absence with prior beta-adrenergic blockade. ISAM Study Group. *Circulation* 1989;80:853- 858.
- [120] Kupari M, Koskinen P, Leinonen H. Double-peaking circadian variation in the occurrence of sustained supraventricular tachyarrhythmias. *Am Heart J.* 1990 Dec;120(6 Pt 1):1364-9.
- [121] Peckova M; Fahrenbruch CE; Cobb LA; Hallstrom AP. Circadian Variations in the Occurrence of Cardiac Arrests: Initial and Repeat Episodes. *Circulation.* 1998; 98:31-39.
- [122] Singh RB, Niaz MA, Cornelissen G, Otsuka K, Siegelova J, Fiser B, Halberg F. Circadian rhythmicity of circulating vitamin concentrations. *Scripta Medica (BRONO),* 2001, 74:93-96.



## NEWS

# Decreased Potential of Mitochondria May Increase Risk of Cardiovascular Disease

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The ability of people to perform aerobic exercise varies. Recent experimental studies with rats indicate that those individuals with a low tolerance for aerobic exercise may have a lot more to worry about than just their inability to run fast and for long distances. The same underlying defect that reduces aerobic capacity may also predispose a person and could increase the possibility of coronary artery disease and strokes.

A research team including Ulrik Wisloff of the Norwegian University of Science and Technology in Trondheim, Sonia Najjar of the Medical College of Ohio in Toledo, and Steven Britton of the University of Michigan, Ann Arbor, reports that rats that have been selectively bred to have reduced capacity for aerobic exercise show obesity, insulin resistance and high blood pressure, all symptoms of the so-called metabolic syndrome that increases the risk of cardiovascular disease. The researchers also provide evidence that impaired function of the mitochondria, small structures that produce most of a cell's energy, underline the metabolic problems of the rats with low aerobic capacity. (*Science*, Jan 21, 2005, 307:418).

Previous work had implicated poor mitochondrial function with individual components of the metabolic syndrome, but this is the first time researchers have linked it to all of them at once, “This is an incredibly provocative study, according to Vamsi Mootha of Massachusetts General Hospital in Boston, whose own work has linked mitochondrial malfunction to type II diabetes. “They linked metabolic syndrome to mitochondria in a way that hasn’t been done before.” The rat-breeding experiments began in 1996, motivated mainly, by dissatisfaction with existing animal models for diabetes and cardiovascular disease. Most of those models were created by very nonphysiological means, such as tying off the coronary artery or administering a drug destroying the beta cells of the pancreas, far removed from the way the conditions develop naturally.

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To produce animals whose diseases more closely mimic those in humans, the researchers selectively bred rats to have either high or low capacity for aerobic exercise. They identified rats with a high capacity to run on a treadmill and mated them with one another, and they did the same for animals with a low running capacity. Since oxygen metabolism is such a large part of biology, defects in it should manifest. The animals described in the current report, the products of 11 generations of selective breeding, have a 350% difference in their running abilities. And by every measure tested, the couch potato rats rank high on the cardiovascular risk factor scale; Compared to high-capacity runners, they are more obese, have higher blood pressures and higher levels of blood fats and have increased insulin resistance.

It is known that obesity itself can decrease aerobic running capacity, a statistical analysis showed that it accounts for no more than 20% of the decreased aerobic capacity. . Indeed, studies of very young rats who were poor exercisers showed that metabolic changes, hyperlipidemia and hyperglycemia occurred before any weight differences became apparent. Rural and urban differences in India also indicated higher

Prevalence of metabolic syndrome in urbans compared to rurals who have enormous physical activity. Because mitochondria provide the energy for

exercise, Britton and his colleagues examined whether these organelles exhibited signs of reduced function in the low-aerobic-capacity rats .The researchers found that muscle from those rats had much lower concentrations of a number of key mitochondrial proteins than did muscle from the high-capacity animals. This indicates that they had either fewer mitochondria or less effective ones. This may be similar to subjects with high aerobic capacity compared to obese people with low capacity. The work provides “a strong link among people who suspect that their own aerobic capacity may be on the low side.” Wisloff’s team is testing whether regular exercise can reduce the various risk factors in the low-aerobic –capacity rats, and early results look promising that aerobic capability depends on mitochondria.

What is most interesting is that RB Singh, Rajeev Kumar from Moradabad, S.S. Rastogi from Delhi and Reema Singh Rao from Mumbai; all from India have demonstrated that treatment with Coenzyme Q10 can decrease the various components of the metabolic syndrome within a period of 12 weeks. Coenzyme Q10 is very rich in mitochondrial cell membrane and responsible for ATP sparing and energy generation. Abstract presented in the 4<sup>th</sup> World Conference of the International Coenzyme Q10 Association, LA, USA, April 14-17,2005,Abstract book,p155).



## NEWS

### Not Only a Cold Climate but Also a Hot Climate Can Trigger Cardiac Events

**Daniel Pella<sup>1</sup>, MD; Dasa Dudova<sup>1</sup>, MD. Rajiv Kumar,<sup>2</sup> MD, and S. B. Gupta,<sup>2</sup> MD**

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Acute myocardial infarction (AMI) is a highly dynamic event, which is associated with marked neuroendoorinological dysfunction in addition to cardiac damage. The immediate trigger for AMI is not precisely known. Studies conducted by Lown, Braunwald, Halberg, Otsuka, and our group have demonstrated a marked increase in sympathetic activity, oxidative stress, and magnesium and potassium deficiency during AMI. Clinical studies have reported an increased incidence of AMI sudden death and ischemia during the first quarter of the day when there is a rapid withdrawal of vagal activity and an increase in sympathetic tone. In one case-control study of 202 patients with AMI, there was a significant ( $P>0.02$ ) increase in cardiac events in the second quarter of the day compared to other quarters, respectively (16.8%, 41.0%,13.8%,28.2% per quarter). This characteristic remained prevalent in both men and women and among patients with and without known AMI (n=52), diabetes (n=53) or hypertension (n=75).Triggers of AMI were noted among 162(82.2%) of the patients, Neuropsychological mechanisms were observed as follows: emotional stress (45.5%), sleep deprivation (27.7%), cold climate (29.2%) , hot climate (24.7%), large meals(47.5%) and physical exertion (31.2%).These triggering factors are known to enhance sympathetic activity and decrease vagel tone, resulting in an increased secretion of plasma cortisol, noradrenaline aldosterone, angiotension-converting enzyme (ACE), interleuking (II.)-1,-2,-6,-18, and timor necrosis factor-alpha (TNF-alpha), all of which are proinflammatory agents . There is also a deficiency in the serum levels of vitamin A, E and C and magnesium, potassium, melatonin, and IL-10 (an anti-inflammatory agent). In the above study, a decrease in magnesium, potassium, vitamin A, E, C and

beta carotene combined with an increase in thiobarbituric acid-reactive substances (TBARS), MDA and diene conjugates, TNF-alpha and IL-6, were observed, all of which are indicators of oxidative

damage and proinflammatory activity, respectively. Hot climate as a trigger of AMI has not been reported in the earlier studies. (*Singh et al, Biomed Pharmacother 2004,58:(Supple)111-115*).



## NEWS

### **Scientific Session News from the American College of Cardiology, 54<sup>th</sup> Annual Scientific Session, Orlando, March 6-9, 2005. Trials Cover Stem Cells, Low LDL, Medication Effectiveness**

**Dasa Dudova, MD, Daniel Pella\*,  
MD and Rafaé Ryber, MD.**

Faculty of Medicine, Safaric  
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Participants and attendees of ACC" 05 took in emerging procedures and technology during four days of late-breaking clinical trials. The following represent just a few trials presented during this year's Scientific Sessions. The ACC Scientific Session presented a late-breaking clinical trial session on small, provocative or experimental trials that delved into the following promising areas of clinical research for the first time this year.

#### **Stem Cells in Acute Myocardial Infarction**

Four-month results from the first double-blind placebo-controlled, randomized trial to evaluate the safety and effectiveness of intracoronary transplantation of autologous bone-marrow-derived mononuclear stem cells into patients with acute myocardial infarction (MI) found that the procedure is safe and capable of reducing infarct size. Sixty-seven patients who had suffered an acute MI and presented for treatment two hours after onset of pain were randomized to receive a stem-cell infusion or a placebo within 24 hours after successful mechanical reperfusion of the infarcted artery. "Bone-marrow cell infused after myocardial infarction can significantly alter the structural adaptation of the heart to transient coronary occlusion -infarct healing", said the study's presenter Stefan Janssens, M.D., University of Leuven, Belgium. The reduction in the compensatory hypertrophy of the remote myocardium in association with the finding suggests a benefit in diastolic function indicating a preventive role of stem cells in heart failure.

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## **Autologous Myoblasts in Ischemic Cardiomyopathy**

Three-year follow-up results from a study evaluating the safety and feasibility of transplanting the autologous myoblasts into 22 patients with ischemic cardiomyopathy undergoing coronary artery bypass grafting showed the procedure to be both safe and feasible for these patients and that efficacy trials are warranted. Patients with a previous MI and left-ventricular ejection fraction of less than 40 percent were enrolled in the trial and injected with skeletal muscle myoblasts in the area of infarction, said Nabil Dib, M.D., director of cardiovascular research, Arizona Heart Institute, Phoenix. Positron-emission tomography and magnetic resonance imaging scans showed evidence of myoblast viability in the area of graft scar tissue, and the patients' ejection fraction improved almost one New York Heart Association (NYHA) functional class.

The results of a small phase I study of the effect of percutaneous transendocardial injection of autologous skeletal myoblast cells on left-ventricular (LV) function in 15 post-MI patients with chronic heart failure indicates that the procedure improves ejection fraction and LV wall motion but is also associated with an increased risk of ventricular arrhythmias. "Randomized, properly powered and closely monitored trials are needed to assess the true safety and efficacy of this treatment in these high-risk patients," said Patrick Serruys, M.D. Chief of Interventional Cardiology, Thorax center, Erasmus University, the Netherlands.

## **Metalloproteinase Inhibitor (PG116800) in AMI**

A randomized experimental study of the effectiveness of a matrix metalloproteinase inhibitor (PG-116800) in preventing LV remodeling in 253 patients with acute MI concluded that at 90 days post-MI, there was no difference in LV remodeling among patients who received a placebo. In addition, 60 percent of the patients had an increase in LV end-diastolic volumes that could potentially lead to heart

failure, according to W. Douglas Weaver, M.D., Henry Ford Heart and Vascular Institute, Detroit.

## **Gene Therapy in Coronary Artery Disease**

The final results of the largest gene-therapy trial to promote myocardial angiogenesis (AGENT-3) using Ad5FGF-4, a replication-deficient, serotype-5-adenovirus-encoding fibroblast growth factor, found that this therapy failed to accomplish the trial's primary endpoint of increasing exercise duration for the 416 patients with class II to IV angina enrolled in the trial. However, researchers from the 65 U.S. centers where the trial was conducted did see some improvement in exercise time in a subset of very high-risk patients, including those with severe class III and IV angina, said Timothy Henry, M.D., director of clinical research, Minneapolis Heart Institute Abbott Northwestern Hospital.

## **BRAVE-2 Trial with Abciximab**

The BRAVE-2 trial, a randomized European multi center trial to assess the value of mechanical reperfusion (percutaneous coronary intervention plus glycoprotein IIb/IIIa inhibition with abciximab) in 365 patients with ST-segment elevation acute MI presenting more than 12 hours after onset of symptoms, demonstrated that this therapy reduces infarct size. The results of the trial support the use of mechanical reperfusion in patients with acute MI who present late, said Adnan Kastrati, M.D., Deutches Herzzentrum (German Heart Centre), Munich, Germany.

## **The CACAF Study**

This prospective, randomized, controlled study of the effect of catheter ablation on atrial fibrillation (AF), showed that catheter-ablation therapy combined with antiarrhythmic drug therapy alone in preventing recurrences of atrial arrhythmias in 137 patients with paroxysmal or persistent AF who had already failed

antiarrhythmics. The patients in the ablation group underwent cava-tricuspid and left-inferior pulmonary vein mitral isthmus ablation plus circumferential pulmonary vein ablation, said Emanuele Bertaglia, M.D., chief of the electrophysiology lab, Civic Hospital of Mirano, Venice, Italy. Ninety-four percent of the patients in the control group had at least one AF recurrence versus 40.6 percents in the ablation group.

## CPAP for Heart Failure

A trial of the use of continuous positive airway pressure (CPAP) for 258 heart-failure patients with central sleep apnea failed to show that CPAP reduces mortality in these patients , according to T. Douglas Bradley , M.D ., Professor of Medicine, University of Toronto. Mortality rates were the same among patients who received CPAP versus those in the

control group. However the CPAP-treated patients did have improvements in LV function and exercise tolerance. In addition, their sleep apnea was alodrepinephrine at 12 weeks follow-up.

## The PEECH Trial for Heart Failure

Finally, in the PEECH trial a prospective evaluation of the use of enhanced external counterpulsation (EECP) in combination with optimal care for 186 heart-failure patients, demonstrated that EECP improves exercise time but not peak VO<sub>2</sub>, said Arthur Feldman, M.D., Ph.D., Chairman, Department of Medicine, Jefferson Medical college, Philadelphia. In addition, patients who received EECP had significant improvements in quality of life and NYHA function class versus patients who received optimal therapy alone.





## BOOK REVIEW

### **Coronary Artery Disease: Risk Promoters, Pathophysiology and Prevention**

Editors; Gundu HR Rao, S Thanikachalam, Publishers, Jaypee Brothers, New Delhi, India.2005, email: jaypee@jaypeebrothers.com

#### **Reviewed by RB Singh, MD**

Professor of Medicine, Subharti Medical College  
Founder President, International College of Cardiology  
Hon Fellow, Halberg Chronobiology Centre, University of Minnesota, Minneapolis, Minnesota, USA

The editors of this book, which assembles world-renowned authorities to discuss most of the areas of cardiology, are among the best in their field. The contents of the book are valuable and its form compares favorably with other books published in India (despite a few typographical lapses such as instructions to the typesetter appearing in print). The book was published in 2005 but references quoted are hardly up to 2002; only some authors publishing in 2003 are being considered. The chapters by Drs U.N. Das, Enas A. Enas, P.C. Manoria, V.S. Narain, Gundu H.R. Rao, Y.K. Seedat and Bela Shah are outstanding. Considerations revolving around diet, sedentary behaviour, tobacco and other loads, associated with coronary artery disease (CAD) are discussed by most of the authors, although more emphasis may be welcome in the future. In any future editions of this book, each author should be persuaded to revise his chapter to quote studies published in the intervening years. There is no chapter on physical activity, dietary prevention and stress management indicating their

role in the pathophysiology and prevention of CAD. In recent years there has been a flurry of activity in developing new pharmaceutical agents that target both conventional and newly discovered pathways in the development of vascular disease. This book has quite authentically discussed the role of low molecular weight heparin, direct thrombin inhibitors, tissue factor pathway inhibitors and of an oral form of unfractionated heparin but some of the authors have not been able to take the task as seriously as desirable for the readers.

Many authors on classical epidemiology and prevention have merely repeated the same studies multiple times. In the future, all experts interested in epidemiology should join hands to write one large chapter on CAD in south Asians and another chapter on CAD among south Asian immigrants to developed countries. The challenge and opportunity of blood pressure chronomics in India by the BIOCOS group is unique in the sense that its implementation avoids the need for fixed limits to the fiction of a "true" blood pressure. The role of alterations in the variability of pressures is important and is an early warning as a desideratum and is probably contributory, if not responsible for CAD and stroke. It can be treated for stroke and other serious CAD and stroke prevention!

With this rare but important emphasis of chronomic (time-structural) studies now under way in Amritsar, Lucknow and Moradabad, the book can be warmly and unreservedly recommended to cardiologists, other physicians, community medicine experts, still other health professionals, cardiac surgeons, nutritionists, biochemists and dietitians and appears to be the best among all the books published on this subject during the last five years from India.

## **Trends in Atherosclerosis Research**

Edited by Leon V.Clark, Nova Biomedical Books, 2004, NY, USA; email, Novascience@earthlink.net, www.novapublishers.com

### **Reviewed Dr Chi-Woon Kong, MD**

President, International College of Cardiology  
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The editor Dr Clark appears to be extremely good in selecting the authors for various chapters included in this book. The book posses the material which is of international standard. Each chapter has been written by a well known expert in the concerned field.

Mechanistic Approaches to Therapy for Vascular Injury, Surgical Myocardial Revascularization: Off Pump or on a Better Pump?. Targeting the CD40-CD40 Ligand (CD154) System as Therapeutic Intervention for the Treatment of Atherosclerosis.

The role of Matrix Metalloproteinases in Atherosclerotic Plaque Instability.

Investigation of the Role of Endogenous Nitric Oxide Synthesis in Determining Patterns of Arterial Wall Permeability and Diet-Induced Lipid Deposition in the Rabbit.

Large Artery Stiffness: Structural and Genetic Aspects. Small Vessel Ischemic Disease and Atherosclerosis: From Pathophysiology to Risk Factors. Pathophysiology and Pathogenesis of Vascular Cognitive Impairment: A Critical Update. Assessment of Reverse Cholesterol Transport. The Therapeutic Possibilities for the Regression of Vascular Changes in Hypertension. Analytical Methods of Atherosclerosis Research are various chapters available in this book. This may be quite interesting to internists, physicians, cardiologist, basic scientist, health workers, pharmacologists and nutritionists. However, role of nutrition in the atherosclerosis has not been dealt adequately.

## **Focus on Atherosclerosis Research**

Edited by Leon V.Clark, Nova Biomedical Books, 2004, NY, USA; email, Novascience@earthlink.net, www.novapublishers.com

### **Reviewed Dr Chi-Woon Kong, MD**

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The authors appear to be the great servants of cardiovascular sciences. The book has provided most interesting material to the basic scientist as well as to clinical cardiologist and health scientists. The Role of Infection in Atherosclerosis . Cardiorespiratory Fitness and Atherosclerotic Cardiovascular Diseases. Childhood Risk Factors Predict Adult Risk Associated with Subclinical Cardiovascular Disease and Strategies for Prevention: The Bogalusa Heart Study. A Role for Chlamydia pneumoniae in Atherosclerosis: Fact or Fictions? Oxidized Low-Density Lipoprotein Complexed with b2-Glycoprotein I as a Common Metabolic Form in Atherogenesis and Autoimmune-Medical Atherosclerosis.

Impact of Lipoprotein Metabolism on Extreme Longevity. Fatty Acids: Determinants of Endothelial Function, Inflammation, Insulin Resistance and Atherosclerosis. Involvement of Endothelial Dysfunction in Atherogenesis. Oxidative Stress Hypothesis of Atherosclerosis. Vascular Oxidant Stress and Endothelial Dysfunction in Hyperhomocysteinemia.

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