Signature: Med Sci Monit, 2003; 9(1): RA1-8
PMID: 12552252

WWW.MEDSCIMONIT.COM
Review Article

RA

Received: 2002.06.17
Accepted: 2002.11.28
Published: 2003.01.28

Biological rhythms, endothelial health and cardiovascular disease

Janie F. Walters[1], Debra J. Skene[2], Shelagh M. Hampton[2], Gordon A.A. Ferns[1,3]

[1] Centre for Clinical Science & Measurement, School of Biomedical and Life Sciences, University of Surrey, Guildford, Surrey, UK
[2] Centre for Chronobiology, School of Biomedical and Life Sciences, University of Surrey, Guildford, Surrey, UK
[3] The Royal Surrey County Hospital, Egerton Road, Guildford, Surrey, UK

Summary

The activity of several components of the vascular system appears to be diurnally regulated. Endothelial cell activation, leukocyte and platelet interactions and lipoprotein metabolism have all been shown to vary with time of day, but whether these variations are due to the endogenous circadian clock, exogenous factors, such as the light-dark cycle, or an interaction between the two remains to be determined.

Endothelium-dependent vasodilation also varies diurnally. This rhythmicity is lost in individuals with established coronary disease has been shown to occur in the early stages of atherosclerosis.

The incidence of coronary events appears to be higher in the early hours of the morning, this may be related to heightened activity of the autonomic nervous system at this time. Higher circulating levels of catecholamines in the morning are associated with increased vascular tone, affecting circulating blood volume and blood pressure. Time dependent variations may be of particular significance for individuals with disrupted circadian rhythms, including rotating shift workers, transmeridian travellers and blind individuals with no light perception.

Variations in endothelial function are observed during the menstrual cycle, varying with circulating oestrogen levels. Oestrogen deficiency in postmenopausal women may contribute to endothelial dysfunction, together with other modifiable risk factors. The absolute risk of coronary disease is greater for men than for pre-menopausal women. Following the menopause gender differences in coronary risk are thought to diminish, although this remains controversial. This review focuses on the influence of both endogenous biological rhythms and environmental factors on the function and health of the human vascular system.

key words: **circadian • endothelial • coronary heart disease**

Full-text PDF: http://www.MedSciMonit.com/pub/vol_9/no_1/2856.pdf
Word count: 3659
Tables: –
Figures: –
References: 89

Author's address: Professor Gordon A.A. Ferns, Centre for Clinical Science & Measurement, University of Surrey, Guildford, Surrey GU2 7XH, UK, email: g.ferns@surrey.ac.uk

BACKGROUND

Disrupted circadian rhythms have been implicated in the genesis of cardiovascular and cerebral disease; and myocardial infarction, cardiac arrhythmias, sudden cardiac death and stroke, have a peak incidence in the morning hours (06:00 to 12:00 hrs) and a lower incidence at night [1]. Studies have also shown that there is a time-dependent change in leukocyte activation and endothelial function, and these may play a critical role in the pathophysiology of thrombotic events and other inflammatory disorders [2]. However, the biological rhythmicity in these functions is modulated by both endogenous and exogenous influences.

Biological rhythms may be circadian (with a periodicity of approximately 24 hours), circatrigintan (such as the menstrual cycle in women) and circannual (seasonal variation brought about by changes in day length). Disruption of biological rhythms may affect several physiological and behavioural functions, directly or indirectly. In mammals, circadian rhythms are endogenously generated by a 'pacemaker' located in the suprachiasmatic nuclei (SCN), a distinct cluster of cells localised within the hypothalamus. More recent studies have shown evidence of peripheral oscillators, however it is generally considered that oscillations generated from peripheral sites are kept '*in phase*' by the SCN oscillator. The pineal gland is a neuroendocrine transducer, which receives information directly from the SCN in response to signals from retinal photoreceptors via the retinohypothalamic tract. In response to information from the retina, the SCN stimulates the pineal to synthesise and secrete the hormones serotonin, during the day, and melatonin at night. The nocturnal rise in melatonin also depends on increased norepinephrine (NE) in sympathetic nerve terminals acting on β- and α-adrenergic receptors [3]. The 'chronobiotic' effects of melatonin (*N*-acetyl 5-methoxytryptamine) have been extensively studied [4]. However, the physiological effects of endogenous melatonin are poorly understood and little is known about its effects within the cardiovascular system.

The effects of melatonin are mediated by binding to specific, high affinity G-protein coupled receptors localised to the plasma membrane. Two mammalian receptor subtypes have been identified, MT_1 (Mel_{1A}) and MT_2 (Mel_{1B}) [5,6]. Recent evidence suggests that melatonin may have a direct influence on vascular function via the MT_1 receptors located on coronary arteries [5]. Many of the physiological factors that affect the vascular system, including plasma epinephrine (adrenaline), tissue plasminogen activator (PA-1), platelet aggregation and fibrinolysis appear to be regulated by the circadian clock, either directly or indirectly [2].

Under natural illumination, and most laboratory lighting conditions, animals are entrained to the 24-hour period of the light-dark cycle [7]. The 'masking' effects of the light-dark cycle or other environmental stimuli may only be excluded in experiments conducted under constant conditions, i.e. temporal isolation from all time cues [8]. Unfortunately, many of the experiments investigating circadian variation in vascular parameters have not accounted for the exogenous effect of environmental influences. Consequently, it is not yet proven that the observed rhythms are indeed endogenously generated (i.e. circadian).

DIURNAL VARIATION OF ENDOTHELIAL FUNCTION AND CORONARY HEART DISEASE

The role of the vascular endothelium

Endothelial cells form the interface between the circulating blood and the artery wall, and are therefore well placed to play a number of important roles [9]. Circulating blood exerts shear forces on the endothelium, inducing flow-mediated dilation. Shear stress has been shown to actively influence vessel wall remodelling [10]; a process that is dependent on intact endothelial function [11]. In response to sheer stress, and other stimuli such as, insulin, endothelin-1, acetylcholine, adenosine diphosphate (ADP), thrombin and bradykinin, the endothelium releases several vasoactive mediators that include nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDHF) and prostacyclin (PGI_2) [12]. NO diffuses from the endothelium, acting within the circulation to inhibit platelet aggregation, and also acts on the underlying vascular smooth muscle to induce relaxation [13]. NO probably also exerts an autocrine/ paracrine effect on the endothelium, by modulating the expression of cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) [14]. This may be particularly important in preventing the early stages of atherogenesis.

Diurnal variation and endothelial dysfunction

Studies have shown time-dependent changes in endothelial function. Shaw et al (2001) [15] demonstrated that in healthy male subjects, endothelium-dependent vasodilation is subject to a time of day variation, which may act as a cardioprotective mechanism to counteract potentially adverse time-dependent changes in haemodynamic parameters including thrombosis and fibrinolysis. Significantly, individuals with hypertension and established CHD lose this diurnal rhythm, and this may contribute to the progression of atherosclerosis [15].

Endothelial dysfunction presents early in the evolution of coronary disease. It is characterised by abnormal vasomotor function, loss of permeability barrier function, adhesion molecule upregulation and a prothrombotic surface [16]. These changes are partially due to an imbalance in the expression of endothelium-derived mediators [16]. The biological activity of endothelium-derived NO is impaired in individuals with hypertension and atherosclerotic disease. Studies investigating the diurnal variation in urinary markers of NO production (nitrate and cyclic GMP) have shown evidence of time-dependent changes [17]. In response to shear stress induced by the flow of blood along the endothelial surface [18,19] and the effect of mediators, such as

bradykinin, serotonin and acetylcholine, NO is continuously released. Physical exercise causes an increase in blood flow and thus increases shear stress, which may also be responsible, in part, for the increased NO release observed during the day [20]. Bode-Boger et al (2000) [17] reported that differences exist in the rhythmic production of NO between healthy individuals, and patients with hypertension or peripheral arterial occlusive disease. Individuals with atherosclerotic disease were found to have a reduced amplitude in the levels of NO produced in comparison with healthy individuals, and the twenty-four hour rhythm was absent in patients with hypertension [17].

Melatonin receptor subtype: expression on endothelial cells?

The expression of the MT_1 melatonin receptor has been localised to the SCN, pars tuberalis of the pituitary, MCF-7 breast cancer cells and coronary vessels [5,6,21]. Evidence for the expression of the MT_1 receptor in human vasculature was derived from Western blotting of sections of isolated coronary artery, obtained from individuals undergoing orthotopic heart transplantation [22]. Receptor expression in these tissues was confirmed by the identification of a band of approximately 39 kDa (relative to a previously predicted molecular mass of 37 kDa) and this was supported by positive findings using RT-PCR analysis.

However, the exact cellular localisation of the receptor was not investigated. The authors also reported evidence of a significant 24-hour variation in receptor expression, with lowest values recorded during the morning hours and peak values detectable in the early evening. This was determined by having the results from the Western blots against the exact time of applying the aortic clamp and the rhythm calculated via cosinor analysis of the data. However, only single clock hour samples were obtained [22]. Gauer et al (1993) [23,24] showed that in the rat pars tuberalis and SCN, melatonin receptor density is inversely proportional to circulating levels of melatonin. Thus, melatonin receptors appear to be directly regulated by melatonin itself, which could in part, explain the observed time-dependent changes seen in human arteries [24].

There is good evidence for the presence of melatonin receptors in the cerebral (circle of Willis at the base of the brain) and caudal arteries in rats [25]. The affinity of ligand for these binding sites is similar to that reported in the SCN and PT [25]. It has been shown that melatonin causes vasoconstriction and reduced blood flow in rat cerebral arteries by directly binding to receptors on smooth muscle cells [26]. In the rat caudal artery, vascular tone is regulated by norepinephrine (NE), originating from sympathetic nerves and melatonin affects vascular tone in these tissues indirectly, by potentiating the effect of NE [27]. Similar studies using porcine coronary arteries, have found that serotonin causes smooth muscle cell contraction, and endothelium-dependent relaxation via the release of NO [28, 29]. Melatonin has been shown to inhibit the contractile response to serotonin in isolated rabbit aorta vascular smooth muscle [30]. The findings suggest that the action of melatonin on serotonin-induced contraction of coronary arteries may interfere with calcium influx from nifedipine-sensitive L-type voltage-sensitive calcium channels (VSCC) [30]. The effect of melatonin on NO-mediated endothelium-dependent relaxation has yet to be studied.

The presence of the MT_1 receptor in human coronary arteries therefore suggests melatonin may have an effect on vascular tone and blood flow following receptor activation [22]. The exact localisation of the melatonin receptor in human coronary arteries also remains to be elucidated.

AUTONOMIC FUNCTION AND CARDIOVASCULAR DISEASE

In the early morning, there is a marked rise in neural and hormonal sympathetic activity [31].

The observed diurnal rhythm of blood pressure coincides with a rhythmic release of catecholamines [32]. These changes in autonomic activity may also modulate plasma volume, vascular tone and blood coagulability [32], and influence the balance between coronary blood supply and oxygen demand. Additionally, the arteries of patients with established coronary disease and endothelial dysfunction are likely to be more sensitive to the constrictor effects of catecholamines, and increased sympathetic nervous activity evident during the early morning [31].

It has been suggested that the SCN modulates autonomic output to most organs of the body [33]. The sympathetic innervation of the pineal gland and neural outflow from brain to adipose tissue were the first demonstrations of SCN-peripheral connections [34]. Studies in rats [35] and humans [36] have shown that ambulatory heart rate is affected by exposure to light in a dose-dependent manner. This suggests a potential role for the retina-SCN pathway in the regulation of autonomic functions.

Apparent absence of circadian rhythmicity of blood pressure

Blood pressure fluctuates with the sleep-wake cycle in humans, with levels being generally higher during the day and lower at night. This is associated with increased sympathetic activity during the waking hours [37]. It is well established that the 24-hour blood pressure profile is affected by both environmental and behavioural factors. Furthermore, studies using constant routine techniques, that minimise the influence of environmental cues, have suggested that in healthy subjects exogenous factors are the sole determinants of the observed rhythms [38,39]. Since the day/night blood pressure rhythm is largely independent of the circadian clock, it shows almost immediate adaptation to shifted phases of activity and sleep [39].

TIME DEPENDENT VARIATION OF ENDOTHELIAL CELL ADHESION MOLECULES AND FIBRINOLYSIS

Endothelial cell adhesion molecules

Cell adhesion molecules (CAMs) are present on the cell membranes of many cells including endothelial cells. They play a central role in the recruitment of the leukocyte inflammatory process. They are released into the circulation as soluble adhesion molecules, following endothelial activation [40].

Both E-selectin and ICAM-I are found on the endothelial cell surface. E-selectin is activated by such inflammatory markers as interleukin-1 (IL-1) and tumour necrosis factor alpha (TNF-α) [41]. It has been suggested that since E-selectin is uniquely expressed on endothelial cells, plasma levels of soluble E-selectin may be an indicator of endothelial cell activation [42]. Studies in young, healthy subjects have shown a time-dependent variation in the plasma levels of soluble (s)E- and (s)ICAM-1. A peak concentration occurs at approximately 12:00 hrs and a nadir at 24:00-04:00 hrs [40].

Diurnal variation of fibrinolytic activity

The proteolytic breakdown of fibrin by plasmin in the blood is activated by the endothelium-derived tissue-type plasminogen activator (tPA) and transiently inhibited by the actions of the irreversible plasminogen activator inhibitor type 1 (PAI-1). Under normal circumstances, free active plasmin is not detectable in the circulation, so the fibrinolytic activity in blood is largely determined by circulating levels of tPA, which is closely regulated by PAI-1 [42]. The discovery that fibrinolytic activity shows a 24-hour variation, was reported by Fearnley and co-workers [43].

Further studies have shown that the activities of tPA and PAI-1 are largely responsible for these day-night changes in blood fibrinolysis [44,45]. Andreotti et al [42] showed that the highest levels of plasma tPA were detectable at 18:00 hrs, falling dramatically to barely detectable levels at 03:00 hrs. Fluctuations in plasma PAI-1 were closely related to those of tPA, with low levels at 18:00 hrs and greatest inhibition at 03:00 hrs [42]. This variation in fibrinolytic activity, with reduced levels in the early morning could favour thrombus formation in the early morning and be associated with the reportedly higher morning incidence of acute thrombotic events [44].

THE MENSTRUAL CYCLE AND CARDIOPROTECTIVE PROPERTIES OF OESTROGEN

Oestrogen receptors are found throughout the arterial tree [46] and physiological levels of oestradiol cause relaxation of coronary arteries and stimulate NO production by endothelial cells [47]. The fluctuations in oestrogen concentrations during the menstrual cycle are reflected in changes in levels of several other circulating factors including nitric oxide, vascular endothelial growth factor, adhesion molecules and homocysteine [48]. A reduction in large vessel endothelial function has also been shown to occur in the late luteal phase [49]. Studies have shown that endothelium-dependent vasodilation of the brachial artery increases from the menstrual to late follicular phase, decreases in the early luteal phase and increases again in the late luteal phase [49,50]. These changes are co-incident with variations in the levels of endogenous oestrogens during the menstrual cycle, which rise during the early stages of the cycle, fall during early luteal development and rise slightly during the remainder of the cycle [50].

In addition, there is increasing evidence that melatonin may directly affect the biological responses to oestrogen. Studies investigating the modulation of oestrogen receptor activity in MCF-7 human breast cancer cells have shown that melatonin can suppress oestrogen receptor-α (ER-α) gene transcription and repress its biological activity [21].

Menopause and increased CHD risk: is ageing the important factor?

Data analysis of mortality rates from all causes, including coronary artery disease, in the UK, show that risk increases inexorably with age in both men and women [51]. It has been argued that, there is no *acceleration* of risk in women at or after 50 years, and no age at which risk in both men and women is the same [52]. Similar findings have been shown for other countries including the USA [51].

However there is a large body of evidence indicating that oestrogen is cardioprotective and therefore it must be considered as a contributor to the difference in CHD trends between men and women. Arterial endothelial function appears to decline at a significantly greater rate in postmenopausal women than men [52]. It is possible that oestrogen deficiency in postmenopausal women contributes to endothelial dysfunction and hence may be responsible, in part, for the increased risk of CHD [53].

The menopause is also associated with a number of other changes in coronary risk factor profile that may affect endothelial function. A significant increase in serum cholesterol precedes the natural menopause by several years [54]. Postprandial lipid metabolism is associated with endothelial function and studies have shown the pre/post-menopausal transition is associated with increased levels of triglycerides and cholesterol [55]. High oestrogen levels are associated with greater fibrinolytic activity and lower PAI-1 levels [56]. Consequently, the menopause is associated with changes in haemodynamic function with increased blood pressure and a decrease in the physiological fall in nocturnal blood pressure, effects that can be reversed by hormone replacement therapy [57,58].

CONDITIONS ASSOCIATED WITH INCREASED CARDIOVASCULAR RISK: POSSIBLE CIRCADIAN INFLUENCE?

Diabetes

Diabetes is an independent risk factor for CHD. Hyperglycaemia and insulin resistance, are considered con-

tributory factors to the development of diabetic macrovascular disease. In both animal and human studies, insulin has been shown to have a direct effect on vascular function. In animal models, insulin has been shown to induce endothelial-derived nitric oxide release [59] and in humans, to stimulate nitric oxide-dependent basal blood flow [60].

Insulin resistance is associated with impaired endothelium-dependent vasodilation [61] and is particularly prevalent in individuals with obesity or type II diabetes [62,63]. It has been suggested that there may be similarities between the signalling pathways responsible for insulin-stimulated glucose uptake and insulin-stimulated endothelial function [64]. High glucose concentrations have been postulated to have a direct effect on endothelial function, via an increased production of vasoconstricting agents (prostaglandins) or increased generation of reactive oxygen species (superoxide anions) [65,66].

It has also been well established that long term treatment with insulin and other hypoglycaemic drugs, improves insulin-stimulated glucose uptake and other metabolic functions [67]. Rask-Madsen et al [68] investigated the effect of two months of treatment with insulin treatment on endothelium-dependent vasodilation in individuals with type II diabetes or ischaemic heart disease. Their results showed a partial restoration of insulin-stimulated vascular function in both groups, although no improvements in endothelium-dependent vasodilation were observed [68].

Studies have shown that diabetic individuals with established coronary artery disease are at increased risk of recurrence compared with non-diabetic coronary patients [69] and that this risk may be associated with a disruption of the normal altered rhythm of autonomic, haemostatic and fibrinolytic functions [45]. Diabetic individuals exhibit a blunted circadian variation of tPA and PAI-1 coupled with platelet hyperaggregability, which persists throughout the day [70]. This sustained prothrombotic state may attenuate the relative protection from thrombotic events during the evening and night-time [45].

However the above studies do not appear to have controlled for exogenous, confounding effects such as the light-dark cycle, such that the precise contribution of the endogenous circadian clock to these phenomenon has not yet been determined.

Shift Work

There is increasing evidence to suggest that shift workers have an increased risk of developing coronary heart disease [71,73]. Maladaption of endogenous circadian rhythms to abrupt changes in shift times are associated with disturbed sleep and meal patterns and may contribute to this risk. Heart rate and blood pressure of shift workers has been shown to increase during work and decrease during sleep, independently of clock time and type of shift (day or night shift) [74].

Since heart rate and blood pressure are under the control of the autonomic nervous system, it is predicted that shift work also alters the circadian rhythm of sympathetic activity [75]. These changes may also be related to the increased cardiovascular risk associated with shift work [71,72]. Studies investigating the effect of shift work on blood pressure, have shown the greatest change in the sleep/wake difference, in mean systolic/diastolic ambulatory blood pressure, occurs in day shift workers, compared to night shift workers, independent of age and body mass index [32].

It is becoming increasingly accepted that triglycerides are an independent risk factor for coronary disease [76]. The predominance of small low-density lipoproteins (LDL) is associated with increased coronary risk, as these particles are more able to infiltrate the arterial wall and are more easily oxidised, leading to enhanced atherogenesis [76]. Elevations in both plasma triglycerides and cholesterol have been reported in some shift workers [77]. Studies investigating the effect of simulated shift work on the postprandial responses to a standardised mixed meal in young healthy subjects, showed that postprandial glucose, insulin and lipid levels are affected by circadian alterations [78,79]. Rotating shift workers are thus likely to demonstrate insulin resistance and altered lipid responses when meals are consumed at the beginning of a period of night-shift work and during daytime shifts, when unadapted [79].

SEASONAL PEAK IN CARDIOVASCULAR MORTALITY

Seasonal change of human circadian rhythms

The circadian clock not only regulates physiological functions in accordance with the twenty-four hour light-dark cycle, but also with the seasonal change in photoperiod [80].

Melatonin plays an important role in the regulation of seasonal rhythms in photoperiodic mammals, being involved in regulating seasonal cycles of reproduction, coat growth, milk production as well as fasting, thermoregulation and hibernation [81]. The duration of the nocturnal melatonin peak changes according to photoperiod, increasing in accordance with the length of the dark period, such as during winter [82].

In contrast, until recently the only seasonal change in humans was a change in the timing of the endogenous melatonin rhythm, being phase advanced in the summer in normal, healthy individuals [83–85]. The ability of early morning bright light to phase advance circadian rhythms [86], may explain why the timing of circadian rhythms is more advanced in summer than winter. However, more recently, in a carefully controlled study exposure of subjects to 8hrs light : 16hrs complete darkness for two months has been shown to increase the duration of melatonin secretion compared with two months exposure to 14hrs light : 10hrs dark [87].

Thus humans, like photoperiodic animals appear to possess the ability to respond to changes in photoperiod.

Increased incidence of CHD during winter

It is well known that short-term climatic changes (e.g. heat wave) transiently influence cardiovascular mortality [87]. It has been suggested that climatic changes during the winter months may induce physiological stresses including sympathetic activation, hypercoagulability and infection, leading to an increased incidence of acute coronary events and stroke [88]. Evidence suggests that the observed seasonal variation in CHD mortality is particularly associated with increasing age [89].

Acute exposure to cold in humans is known to be associated with the stimulation of several autonomic responses including peripheral vasoconstriction, shivering, increased heart rate and blood pressure and particularly in the elderly, whose coronary circulation is already compromised, the increased demand in oxygen consumption could lead to ischaemia, and therefore angina pectoris or myocardial infarction.

The discovery of melatonin receptors in human coronary arteries, and melatonin's role in seasonality, suggests that there may be a physiological basis to the increased incidence of coronary events during winter. More studies are needed to investigate this further.

CONCLUSIONS

It appears that several factors involved in the development of cardiovascular disease are temporally modulated. Blood pressure, vascular tone, lipid metabolism, platelet and leukocyte reactivity and fibrinolysis vary with time of day, but it is unclear whether this is due to an intrinsic rhythm, or driven by environmental cues. There is however emerging evidence that the hormone melatonin may contribute in part to the diurnal variation in vascular function, as receptors for it are present in the coronary circulation. The physiological importance of control by the circadian clock remains to be established. The attenuation of diurnal variation in vascular function, found in association with coronary risk factors, and the potential disturbance of these biological rhythms during shift work may further contribute to cardiovascular risk, and requires further evaluation.

REFERENCES:

1. 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
2. House SD, Ruch S, Koscienski III WF et al: Effects of the circadian rhythm of corticosteroids on leukocyte-endothelium interactions in the am and pm Life Sci, 1997; 60: 2023-2034
3. Reiter RJ: Normal patterns of melatonin levels in the pineal gland and body fluids of humans and experimental animals. J Neural Transm, 1986; 21: 35-54
4. Arendt J, Skene DJ, Middleton B et al: Efficacy of melatonin treatment in jet lag, shift work and blindness J Biol Rhythms, 1997; 12: 604-617
5. Ekmekcioglu C, Haslmayer P, Philipp C et al: Expression of the MT1 melatonin receptor subtype in human coronary arteries. J Recept Signal Transduc Res, 2001; 21: 85-91
6. Vanecek J: Cellular mechanisms of melatonin action. Physiol Rev, 1998; 78: 687-721
7. Armstrong SM, Cassone VM, Chesworth MJ et al: Synchronisation of mammalian circadian rhythms by melatonin. J Neural Transm, 1986; 21: 375-394
8. Wever RA: Characteristics of circadian rhythms in human functions. J Neural Transm, 1986; 21: 323-373
9. Vogel RA, Corretti MC: Estrogens, Progestins, and Heart Disease. Circulation, 1998; 97: 1223-1226
10. Langille BL, O'Donnell F: Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependant. Science, 1986; 231: 405-407
11. Malek AM, Alper SL, Izumo S: Hemodynamic shear stress and its role in atherosclerosis. JAMA, 1999; 282: 2035-2042
12. Vane JR, Anggard EE, Botting RM: Regulatory functions of the vascular endothelium. N Engl J Med, 1990; 323: 27-36
13. Sase K, Michel T: Expression and regulation of endothelial nitric oxide synthase. Trends Cardiovasc Med, 1997; 7: 28-37
14. Khan BV, Harrison DG, Olbrych MT, Alexander W: Nitric oxide regulates vascular cell adhesion molecule 1 gene expression and redox sensitive transcriptional events in human vascular endothelial cells. Proc Natl Acad Sci USA, 1996; 93: 9114-9119
15. Shaw JA, Chin-Dusting JPF, Kingwell BA, Dart AM: Diurnal variation in endothelium-dependant vasodilatation is not apparent in coronary artery disease. Circulation, 2001; 103: 806-812
16. Sagripanti A, Carpi A: Antithrombotic and prothrombotic activities of the vascular endothelium. Biomed Pharmacother, 2000; 54: 107-111
17. Bode-Boger SM, Boger RH, Kielstein JT et al: Role of endogenous nitric oxide in circadian blood pressure regulation in healthy humans and patients with hypertension or atherosclerosis J Invest Med, 2000; 48: 125-132
18. Gnasso A, Carallo C: Association between wall shear stress and FMD in healthy men Atherosclerosis, 2001; 156: 171-176
19. Stepp DW, Merkus D: NO limits coronary vasoconstriction by a shear-stress dependant mechanism. Am J Physiol, 2001; 281: 796-803
20. Maeda S, Miyauchi T, Kakiyama T et al: Effects of exercise training of 8 weeks and detraining on plasma levels of endothelium-derived factors, endothelin-1 and nitric oxide, in healthy young humans. Life Sciences, 2000; 69: 1005-1016
21. Hill SM, Collins A, Kiefer TL: The modulation of oestrogen receptor-alpha activity by melatonin in MCF-7 human breast cancer cells. Eur J Cancer, 2000; 36: 117-118
22. Ekmekcioglu C, Haslmayer P, Philipp C et al: 24h variation in the expression of the mt1 melatonin receptor subtype in coronary arteries derived from patients with coronary heart disease Chronobiol Int, 2001; 18: 973-985
23. Gauer F, Masson-Pevet M, Skene DJ et al: Daily rhythms of melatonin binding sites in the rat pars tuberalis and suprachiasmatic nuclei; evidence for a regulation of melatonin receptors by melatonin itself. Neuroendocrinol, 1993; 57: 120-126
24. Gauer F, Masson-Pevet M, Pevet P: Melatonin receptor density is regulated in rat pars tuberalis and suprachiasmatic nuclei by melatonin itself. Brain Res, 1993; 602: 153-156
25. Laitinen JT, Saavedra JM: Characterization of melatonin receptors in the rat suprachiasmatic nuclei: modulation of affinity with cations and guanine nucleotides. Endocrinol, 1990; 126: 2110-2115
26. Viswanathan M, Scalbert E, Delagrange P et al: Melatonin receptors mediate contraction of a rat cerebral artery Neuroreport, 1997; 8: 3847-3849
27. Viswanathan M, Laitinene JT, Saavedra JM: Expression of melatonin receptors in arteries involved in thermoregulation Proc Natl Acad Sci 1990; 87: 6200-6203
28. Yang Q, Scalbert E, Delagrange P, Vanhoutte PM, O'Rouke ST: Melatonin potentiates contractile response to serotonin in isolated porcine coronary arteries, Am J Physiol, 2001; 280: 76-82
29. Richard V, Tanner FC, Tschudi M, Luscher TF: Different activation of L-arginine pathway by bradykinin, serotonin, and clonidine in coronary arteries. Am J Physiol, 1990; 259: 1433-1439
30. Satake N, Shibata S, Takagi T: The inhibitory action of melatonin on the contractile response to 5-hydroxytryptamine in various isolated vascular smooth muscles. Gen Pharmacol, 1986; 17: 553-558
31. Selwyn AP, Raby K, Vita JA et al: Diurnal rhythms and clinical events in coronary artery disease Postgrad Med J, 1991; 67: 44-47

32. Yamasaki F, Scwartz JE, Gerber LM: Impact of shift work and race/ethnicity on the diurnal rhythm of blood pressure an catecholamines. Hypertension, 1998; 32: 417-423
33. Buijs RM, Hermes MH, Kalsbeek A: The suprachiasmatic nucleus-paraventricular nucleus interactions: a bridge to the neuroendocrine and autonomic nervous system. Prog Brain Res: 1998; 119: 365-382
34. Bartness TJ, Song CK, Demas GE: SCN efferents to peripheral tissues: implications for biological rhythms. J Biol Rhythms, 2001; 16: 196-204
35. Scheer FAJL, Ter Horst GJ, Van der Vliet J, Buijs RM: Physiological and anatomic evidence for regulation of the heart by suprachiasmatic nucleus in rats Am J Physiol, 2001; 280: 1391-1399
36. Scheer FA, van Doornen LJ, Buijs RM: Light and diurnal cycle affect human heart rate: possible role for the circadian pacemaker J Biol Rhythms, 1999; 14: 202-212
37. Baumgart P: Circadian rhythm of blood pressure: internal and external time triggers Chronobiol Int, 1991; 8: 444-450
38. Van Dongen HPA, Maislin G, Kerkhof GA: Repeated assessment of the endogenous 24-hour profile of blood pressure under constant routine. Chronobiol Int, 2001; 18: 85-98
39. Kerkhof GA, Van Dongen HPA, Robbert AC: Absence of endogenous circadian rhythmicity in blood pressure? Am J Hypertens, 1998; 11: 373-377
40. Maple C, Kirk G, McLaren M et al: A circadian variation exists for soluble levels of intercellular adhesion molecule-I and E-selectin in healthy volunteers. Clin Sci, 1998; 94: 537-540
41. Bevilacqua MP: Endothelial-leukocyte adhesion molecules Ann Rev Immunol, 1993; 11: 767-804
42. Andreotti F, Kluft C: Circadian variation of fibrinolytic activity in blood. Chronobiol Int, 1991; 8: 336-351
43. Fearnley GR, Balmforth G, Fearnley E: Evidence of a diurnal fibrinolytic rhythm; with a simple method of measuring natural fibrinolysis. Clin Sci, 1957; 16: 645-650
44. Irokawa M, Nishinaga M, Funayama H et al: Effect of a change in the sleep/wake cycle on the diurnal variation of fibrinolytic parameters. J Thromb Thrombolysis, 1998; 5: 165-168
45. Manfredini R, Gallerani M, Portaluppi F, Fersini C: Relationships of the circadian rhythms of thrombotic, ischemic, hemorrhagic and arrhythmic events to blood pressure rhythms. Annal NY Acad Sci, 1996; 783: 141-158
46. Willekes C, Hoogland HJ, Keizer HA et al: Female sex hormones do not influence arterial wall properties during the normal menstrual cycle. Clin Sci, 1997; 92: 487-491
47. Palacios S: Current perspectives on the benefits of HRT in menopausal women. Maturitas, 1999; 33: 1-13
48. Williams MRI, Westerman RA, Kingwell BA et al: Variations in endothelial function and arterial compliance during the menstrual cycle. J Clin Endocrinol Metab, 2001; 86: 5389-5395
49. Hashimoto M, Akishita M, Eto M et al: Modulation of endothelium-dependant flow-mediated dilatation of the brachial artery by sex and menstrual cycle. Circulation, 1995; 92: 3431-3435
50. Kawano H, Motoyama K, Hirashima O et al: Menstrual cycle variation of endothelium-dependant vasodilation of the brachial artery: possible role of estrogen and nitric oxide. Proc Assos American Physicians, 1996; 108: 473-480
51. Tunstall-Pedoe H: Myth and paradox of coronary risk and the menopause. Lancet, 1998; 351: 1425-1427
52. McCrohon JA, Woo KS, Celermajer DS: A comparison of endothelial function in Caucasian and Chinese women before and after the menopause. Maturitas, 2000; 35: 31-37
53. Virdis A, Ghiadoni L, Pinto S et al: Mechanisms responsible for endothelial dysfunction associated with acute estrogen deprivation in normotensive women. Circulation, 2000; 101: 2258-2263
54. Akahoshi M, Soda M, Nakashima E et al: Effects of menopause on trends of serum cholesterol, blood pressure, and body mass index. Circulation, 1996; 94: 61-66
55. Jensen J, Nilas L, Christiansen C: Influence of menopause on serum lipids and lipoproteins. Maturitas, 1990; 12: 321-331
56. Gebara OC, Mittleman MA, Sutherland P et al: Association between increased estrogen status and increased fibrinolytic potential in the Framingham Offspring Study. Circulation, 1995; 91: 1952-1958
57. Amigoni S, Morelli P, Parazzini F, Chatenoud L: Determinants of elevated blood pressure in women around menopause: results from a cross-sectional study in Italy. Maturitas, 2000; 34: 25-32
58. Butkevich A, Abraham C, Phillips RA: Hormone replacement therapy and 24hr blood pressure profile of postmenopausal women. Am J Hyperten, 2000; 13: 1039-104
59. Chen YL, Messia EJ: Dilation of isolated skeletal muscle arterioles by insulin is endothelium dependant and nitric oxide mediated Am J Physiol, 1996; 270: 2120-2124
60. Scherrer U, Randin D, Vollenweider L, Nicod P: Nitric oxide release accounts for insulin's vascular effects in humans. J Clin Invest, 1994; 94: 2511-2515
61. Baumgartner-Parzer SM, Waldhausl WK: The endothelium as a metabolic and endocrine organ: its relation with insulin resistance. Exp Clin Endocrinol Diabetes, 2001; 109: 1821-1828
62. Baldewg SE, Pink AM: The relationship between obesity, vascular reactivity and endothelial dysfunction in subjects with non-insulin dependant diabetes mellitus. Int J Obes Relat Metab Discord, 2000; 24(Suppl 2): 134-135
63. Serrano Rios M: Relationship between obesity and the increased risk of major complications. Eur J Clin Invest, 1998; 28(Suppl 2): 14-17
64. Cleland SJ, Petrie JR, Small M et al: Insulin action is associated with endothelial function in hypertension and type 2 diabetes. Hypertension, 2000; 35: 507-511
65. Marfella R, Quagliano D, Nappo F et al: Acute hyperglycaemia induces an oxidative stress in healthy subjects. J Clin Invest, 2001; 108: 635-636
66. Graier WF, Posch K, Wascher TC, Kostner GM: Role of superoxide anions in changes of endothelial vasoactive response during acute hyperglycaemia. Horm Metab Res, 1997; 29: 622-626
67. Bolli GB: Physiological insulin replacement in type I diabetes mellitus. Exp Clin Endocrinol Diabetes, 2001; 109(Suppl 2): 317-332
68. Rask-Madsen C, Ihlemann N, Krarup T et al: Insulin therapy improves insulin-stimulated endothelial function in patients with type 2 diabetes and ischaemic heart disease. Diabetes, 2001; 50: 2611-2618
69. Takazoe K, Ogawa H, Yasue H: Increased plasminogen-activator-inhibitor activity and diabetes predict subsequent coronary events in patients with angina pectoris. Ann Med, 2001; 33: 206-212
70. Aronson D, Weinrauch LA, D'Elia JA et al: Circadian patterns of heart rate variability, fibrinolytic activity and hemostatic factors in type 1 diabetes mellitus with cardiac autonomic neuropathy. Am J Cardiol, 1999; 84: 449-453
71. Murata K, Yano E, Shinozaki T: Impact of shift work on cardiovascular functions in a 10 yr follow-up study. Scand J Work Environ Health, 1999; 25: 272-277
72. Akerstedt T, Knuttson A: Cardiovascular disease and shift work. Scand J Work Environ Health, 1997; 23: 241-242
73. Knuttson A: Shift work and coronary heart disease. Scan J Soc Med, 1989; 44(Suppl): 1-36
74. Goto T, Yokoyama K, Araki T et al: Identical blood pressure levels and slower heart rates among nurses during night work and day work. J Hum Hypertens, 1994; 8: 11-4
75. Furlan R, Barbic F, Piazza S et al: Modifications of cardiac autonomic profile associated with a shift schedule of work. Circulation, 2000; 102: 1912-1916
76. Griffin BA: Lipoprotein atherogenicity: an overview of current mechanisms. Proc Nutr Soc, 1999; 58: 163-169
77. Lund J, Arendt J, Hampton SM et al: Postprandial hormone and metabolic responses among shift workers in Antarctica. J Endocrinol, 2001; 171: 557-564
78. Ribeiro DCO, Hampton SM, Morgan L et al: Altered postprandial hormone and metabolic responses in a simulated shift work environment. J Endocrinol, 1998; 158: 305-310
79. Hampton SM, Morgan LM, Lawrence N et al: Postprandial hormone and metabolic responses in simulated shift work. J Endocrinol, 1996; 151: 259-267
80. Broadway JW, Arendt J: Seasonal and bright light changes of the phase position of the human melatonin rhythm in Antarctica. Arctic Med Res, 1988; 7: 201-203
81. Arendt J: Melatonin and the Mammalian Pineal Gland, 1st Edition. Chapman and Hall, 1995

82. Arendt J: Role of the pineal gland in seasonal reproductive function. Oxford Rev Rep Physiol, 1986; 8: 266-320

83. Bojkowski CJ, Arendt J: Annual changes in 6-sulphatoxymelatonin excretion in man Acta Endocrinol (Copenh). 1988; 117: 470-476

84. Arendt J: Melatonin and the pineal gland: influence on mammalian seasonal and circadian physiology. Rev Reprod, 1998; 3: 13-22

85. Buresova M, Dvorakova M, Zvolsky P, Illnerova H: Human circadian rhythm in serum melatonin in short winter days and in simulated artificial long days. Neurosci Lett, 1992; 136: 173-176

86. Czeisler CA, Kronauer RE, Allan JS et al: Bright light induction of strong (type 0) resetting of the human circadian pacemaker. Science, 1989; 244: 1328-1333

87. Wehr TA: The durations of human melatonin secretion and sleep respond to changes in daylength. J Clin Endocrinol Metab, 1991; 73: 1276-1280

88. Douglas AS, Dunnigan MG, Allan TM, Rawles JM: Seasonal variation in coronary heart disease in Scotland. J Epidemiol Community Health, 1995; 49: 575-582

89. Sheth T, Nair C, Muller J, Yusuf S: Increased winter mortality from acute myocardial infarction and stroke: the effect of age. JACC, 1999; 33: 1916-1919