THE DAILY RHYTHM OF BRAIN BLOOD FLOW

By

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Abstract

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By

Deirdre Ann Conroy

Advisor: Professor Arthur J. Spielman, Ph.D.

CBFV (cerebral blood flow velocity) is lower in the morning than in the afternoon and evening. Two hypotheses have been proposed to explain the time of day changes in CBFV. First, CBFV changes are an evoked effect secondary to sleep associated processes, and second, time of day change in CBFV is due to an endogenous circadian rhythmicity driving CBFV lower at night independent of sleep. There is also a greater incidence of strokes, in the morning hours between 6 a.m. and 12 p.m. The aim of the study was to examine CBFV over 30 hours of sustained wakefulness to determine whether CBFV exhibits fluctuations associated with time of day. Nine subjects underwent a modified constant routine protocol. CBFV from the middle cerebral artery was monitored by chronic recording of Transcranial Doppler (TCD) ultrasonography. Other dependent variables included core body temperature (CBT), end-tidal CO2, blood pressure, and heart rate. Salivary dim light melatonin onset (DLMO) served as a measure of endogenous circadian phase position. A non-linear multiple regression, cosine fit

analysis revealed that both the CBT and CBFV rhythm fit a 24 hour rhythm (R^2=.79 and R^2=.50, respectively). FFT analysis revealed that all subjects showed a cycle of ~ 24 hours in CBT and in seven out of nine subjects for CBFV. Salivary DLMO occurred 6-10 hours before CBT nadir in seven out of nine subjects. Aligning both rhythms revealed a 90 degree difference, though not significant (t=-0.52, p=0.62), between the CBT and CBFV rhythm. Once aligned, the rhythm of CBFV closely tracked the rhythm of CBT as demonstrated by the substantial correlation between these two measures (r=.76, p=<.01). In conclusion, time of day variations in CBFV have an approximately 24 hour rhythm under constant conditions, suggesting regulation by a circadian oscillator. The 90 degree-phase angle difference between the CBT and CBFV rhythms may help explain alertness in the evening before habitual bedtime and may contribute to sleep inertia in the morning after waking up.

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I cannot express how deeply thankful I am to Kevin Kerwin. You have been supportive, encouraging, patient, and tolerant throughout this entire grueling process. Your ability to see black and white through life's colorful dramas has been a true asset to this experience. You have been a breath of fresh air, an anchor, and a light at the end of the tunnel. Thank you for being such a precious part of my life.

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Abbreviations

CBFV Cerebral blood flow velocity

MI Myocardial infarction

SCD Sudden cardiac death

CVA Cerebrovascular accident

TIA Transient ischemic attack

CBT core body temperature

SCN Suprachiasmatic nucleus

CR Constant routine

BP Blood pressure

TCD Transcranial Doppler

PET Positron Emission Tomography

Introduction

It has been demonstrated that that cerebral blood flow velocity (CBFV) is lower shortly after awakening in the morning than in the afternoon or evening (Risberg and Ingvar 1972; Townsend et al 1973;Sakai et al 1979; Sakai et al 1980;Meyer et al 1981; Gozukirmiai et al 1982; Heiss et al 1985;Meyer et al 1987; Lenzi et al 1987; Franck et al 1987; Maquet et al 1988;Sawaya and Ingvar 1989; Buchsbaum et al 1989;Sakai et al 1990;Madsen et al 1991; Maquet et al 1990; Droste et al 1993; Hajak et al 1994). To date, no study has properly addressed the question of whether or not this pattern reflects circadian rhythmicity or whether it is a sleep-wake-dependent process (i.e. homeostatic) process that tracks the duration of wakefulness.

CIRCADIAN RHYTHMS

Circadian rhythmicity refers to fluctuations with a cycle or period length of about 24 hours that are internally or endogenously generated. That is, the parameter does not require external time cues to drive the near 24-hour periodicity. A consistent change over 24- hours in a physiological parameter suggests, but does not prove, that the variable is a regulated circadian rhythm. Many physiological systems such as endocrine, gastrointestinal, cardiac, pulmonary, renal, and neurological, show circadian rhythms (for review, see Moore-Ede et al., 1983). Circadian rhythms in mammals are governed by the suprachiasmatic nuclei (SCN) of the hypothalamus. This SCN is the physiological clock that independently generates a near 24-hour output. It receives resetting cues principally from the external light-dark cycle, which keeps the clock of the SCN and the systems it regulates running at a period of 24 hours. The evidence of the effects of light dark cycle

on circadian rhythms and systems that are regulated by circadian rhythms has been well documented.

Physiological functions that are not generated by the SCN are regulated by a "homeostatic process," one that functions according to the duration of prior wakefulness and is largely dependent on the sleep process (Daan et al 1984; Borberly, 1982). Slow wave activity in the electroencephalogram (EEG) during sleep is an example of a homeostatic process because it decreases across the sleep period and increases as a function of wakefulness (Dijk et al 1987). Many functions are governed by an interaction of homoestatic and circadian mechanisms, such as timing of sleep and wakefulness, sleep propensity, and sleep structure (Dijk and Czeisler in 1995).

The generally accepted theory about the time of day changes in CBFV attributes the fall in CBFV to the physiological processes of the sleep period. This theory suggests that the low CBFV in the morning may be a result of the fall in the overall reduced metabolic level (Meyer et al, 1980; Klingelhofer et al, 1992; Hajak, et al. 1994) reduced cognitive processing (Buchsbaum et al. 1989), and decreased cerebral vascular reactivity (Meadows et al 2003) characteristic of the NREM sleep state. Additionally, the reduced physical activity (Diamant et al 2002), reduced body temperature and the recumbent sleeping position have also been proposed as contributions (Yan et al 1997).

The hypothesis of this study is that time of day change in CBFV is due to circadian rhythmicity and is generated by the SCN. To test this hypothesis, the present study continuously monitored CBFV on a ~30 hour constant routine protocol. The purpose of the study was to unmask and quantify contributions of the circadian system and wake dependent influences of CBFV.

B. CBFV AND SLEEP

1. RESEARCH STUDIES

One of the challenges in obtaining CBFV values in sleep is obtaining a recording without disturbing the sleeper. Many different techniques have been used to assess how CBFV changes across the sleep period. Preliminary studies on CBFV in sleep used the "nitrous oxide method" (Mangold et al 1955) as well as the "heat clearance method" (Seylaz et al 1975) and found that CBFV increased in non-REM sleep compared to waking levels.

However, alternative methods revealed different results. Techniques including inhalation of Xenon (Townsend et al 1973; Sakai et al 1979; Sakai 1980; Sakai et al 1990; Meyer 1981; Gozukirmizi 1982 ;Meyer et al 1987), injection of Xenon (Madsen et al 1991), PET scan (Heiss et al 1985; Maquet et al 1988; Buchsbaum et al 1989;Maquet et al 1990; Madsen et al 1991) and more recently Transcranial Doppler ultrasonography (Klingelhoeffer et al 1992; Droste et al 1993;Hajak et al 1994; Hajak et al 1996; Kuboyama et al 1997) have all clearly shown that CBFV decreases in non-REM sleep and increases in REM sleep.

2. CLINICAL STUDIES

A steady CBF is vital to sustain cardiovascular and cerebrovascular health. The heart is responsible for pumping blood to the brain at the rate of about 750-ml per minute and accounts for 15-17% of cardiac output. The brain is one of the most metabolically active organs in the body and relies heavily on a steady supply of glucose and oxygen (20% of the oxygen utilized by the body) from the blood to meet its high-energy

demands. The brain uses only glucose as its energy source but cannot store it. Therefore it must be supplied continuously with a steady flow of blood through the blood vessels. The arteries within the brain play a particularly important role in the blood supply to the brain. The brain is highly susceptible to damage if there is any occlusion of the arteries which may result in infarction (death of the tissue that is supplied by the artery) and can cause brain damage.

A. Myocardial infarction, ischemia and sudden cardiac death

There is a greater incidence of the various types of myocardial infarction (MI) (Muller et al. 1985; Thompson et al 1985; Rocco et al 1987; Rocco 1987b;Thompson et al 1991;Chiang et al 1999;Trappolini et al 2001;Bhalla et al 2001; Kinjo et al 2001; Khan and Ahmad, 2003), transient myocardial ischemic attacks (TIA) (Fox and Mulcahy 1990), and sudden cardiac death (SCD) (Guagnano et al 2000) in the morning hours than in the afternoon or evening. Meta-analyses show that between 6 AM and noon, there is a 40% higher risk of heart attack and a 29% higher risk of cardiac death (Elliot 2001). As of 2003, more than 300 references have confirmed that most cardiovascular physiological parameters (such as heart rate, blood pressure, electrocardiogram indices) and pathophysiological events (myocardial ischemia/infarction, sudden cardiac death) display circadian rhythms (Guo and Stein, 2003).

The first major study on the rhythms in myocardial infarction was The Multicenter Investigation of Limitation of Infarct Size (The MILIS study) (Muller et al. 1985). By examining both the onset of plasma creatine kinase MB (CK-MB) activity to define and the documentation of the onset of pain, this study showed a marked circadian rhythm in the frequency of onset of myocardial infarction, peaking from 6 a.m. to noon. The rhythm of creatine kinase showed peaks at 9 a.m. and troughs at 11 p.m. The variation in these levels also correlated with pain-estimated onset (correlation of 0.85).

Toffler et al. (1987) assessed three separate groups of healthy individuals in a morning study, a 24-hour study, and a delayed arising time study. In the morning study, there was a significant increase in platelet aggregability (measured by ADP and epinephrine) between 8 and 9:30 am. The 24- hour study confirmed the presence of a significant morning increase in platelet aggregability between 6 and 9 a.m. The delayed rise time group served as the author's attempt to disentangle activity-induced activation from the underlying sympathetic activation. At 9:30 am, when the subjects arose from sleep, platelet aggregability was significantly higher. Results from this study suggested that components of awakening (assuming upright posture and beginning the day's activities) did contribute to increased morning aggregability.

ST segment depression during 24-hour EKG monitoring in patients with coronary artery disease was seen in almost half (46%) of the total ischemic episodes occurring between the hours of 6:00 a.m. and 12:00 p.m. (Rocco et al. 1987a). An increase in symptomatic and asymptomatic ischemic ST segment depression was found in the first one to three hours after awakening. Additionally, the heart rate threshold for ischemic ST segment depression was more likely to trigger ischemia in these morning

hours than in the evening hours (Rocco et al. 1987b). Kozak, Krivan, and Semrad 2003 examined patients with implantable cardioverter defibrillators over three months. Patients showed a morning peak in ventricular tachyarrhythmias between 7:00 and 11:00 AM and an afternoon peak between 6:00 and 7:00 PM. A significantly lower occurrence of VT was observed at 1:00 AM and between 4:00 and 6:00 AM. A circadian distribution in the occurrence of ventricular tachycardias was also found.

The current theory of the origin of the morning rise in the incidence of myocardial infarction and sudden cardiac death is that it is related to a combination of endogenous factors (increase in platelet aggregability and blood pressure) and exogenous factors (morning increases in activity i.e. the assumption of the upright position).

The general hypothesis of the triggering of coronary thrombosis in the morning emphasizes behavioral daily activities. The morning activity could contribute to coronary thrombosis by first, physical or mental stress producing hemodynamic changes that rupture a "vulnerable atherosclerotic plaque." Second, an increase in activity may cause coagulability and trigger growth of the thrombus or vasoconstriction. Finally, physical exertion, unstable angina, and cigarette smoking may also cause vasoconstriction, leading to myocardial infarction or sudden cardiac death Muller et al. (1989; 1999).

An alternative theory by Singh et al (2002) suggested that exogenous factors, such as lifestyle or diet, stimulate the SCN to activate the pineal gland and pituitary function which thereby disturbs neurohumoral factors important in the pathogenesis cardiovascular disease.

B. Cerebrovascular accidents

Recent meta-analyses have found a 49% increased risk of strokes between 6 AM and noon (Elliot, 2001). Meta analysis of subarachnoid hemorrhage (SAH) demonstrated the highest occurrence in the period between 6 am and 12 pm (RR = 3.19; 95% CI 3.03-3.36; early morning as a reference variable) and between 12 p.m. and 6 p.m. (RR = 2.63; 95% CI 2.47-2.80 (Feigin et al 2002). It has been suggested that the occurrence of some major acute vascular events (total ischemic strokes, intracerebral hemorrhage, and myocardial infarction) may be influenced by common triggering factors (Feigin 2002).

The increased frequency of the incidence of stroke has been found in the different subtypes of strokes. Incidences of subarachnoid hemorrhage (SAH), intracerebral hemorrhage (IH), and thromboembolic hemorrhage (TH) are higher in both men and women (ages <65 and >65 in Feigin et al 2001 and over age 70 Tsementzis et al. in 1985) between the hours of 10:00 am and 12:00 pm. This has been attributed to the changes in blood pressure. The lowest incidences of all strokes occurred between 12:00 a.m. and 6:00 a.m, when blood pressure falls. Tsementzis et al. (1985) and Wroe et al (1992) found an unusual second peak in the early evening (between 6:00pm-8:00 pm) of SAH, but Feigin et al 2001 did not. The relationship between the increased incidence of stroke in the morning and the concomitant surge in blood pressure in the morning is thought to be due in part to autoregulation. Tsementzis et al. (1985) posit that either the brain is overresponding to a surge in pressure or underresponding, which may also result in subarachnoid or cerebral hemorrhage. They found that more physically intense events, such as sporting or sexual activities, most strongly predicted subarachnoid hemorrhages

while less intense events such as driving, or sleeping predicted intracerebral hemorrhages and cerebral infarction.

Studies have also shown a variability of autoregulation with respect to the time of day. Cerebral vasomotor reactivity in response to hypercapnia (Ameriso et al. 1994; Qureshi et al. 1999) and normal room air (Qureshi et al. 1999) measured via Transcranial Doppler has been found to be lower in the morning (6-8 am) than in the afternoon (1-3 pm) or evening (7-9 pm).

3. SLEEP APNEA STUDIES

Sleep apnea syndrome (SAS) is a sleep disorder characterized by the reductions or cessations of breathing during sleep that are punctuated by awakenings. Following an apneic event (a cessation or reduction of breathing of ten seconds or more) there are typically oxyhemoglobin desaturations (hypoxia), increases in CO2 (hypercapnia) and alterations of blood pressure.

Because these three parameters constitute the major regulators of CBFV, the question was raised of whether SAS patients have impaired brain perfusion. Klingelhoeffer et al (1992) used Transcranial Doppler ultrasonography (TCD) simultaneously with EEG to compare changes in CBFV in sleep of SAS patients to normal volunteers. SAS patients showed a hypersensitivity of CO2 to apnea that depended on sleep stage (highest in REM sleep). However, the pattern of CBFV across the night in SAS patients was similar to normal volunteers. Both groups showed decline in CBFV in non-REM sleep and increases in REM sleep, consistent with previous findings in healthy volunteers. SAS patients demonstrated decreased CBFV values in the

first sleep cycle despite rises in blood pressure and CO2. This study revealed two important findings. First, the autoregulatory mechanisms of SAS patients are intact and their CBFV values were similar to normal healthy volunteers in sleep. Second, decreases in CBFV values in SAS patient occurred in the first sleep cycle despite minimal slow wave sleep. These findings suggested a decoupling of cerebral electrical activity and cerebral perfusion during non-REM sleep.

An additional study from the same group (Hajak et al 1994) further investigated the CBFV and cerebral electrical activity in sleep. Again, simultaneous continuous TCD and EEG were used. In the first sleep cycle (the first third of the night), CBFV values declined coupled with deepening stages of sleep. However, in the second and final cycles of the night CBFV continued to decline despite lightening sleep stages. For example, there were lower values at the end of stage II sleep than in previous slow wave sleep. CBFV values did not change significantly when subjects were briefly awakened. Moreover, there was a delayed rise in CBFV values when subjects were awakened in the morning. The authors suggested that a "central neurogenic control mechanism" may be regulating cerebral perfusion.

A third study using TCD and EEG, Hajak et al. (1996), again compared changes in CBFV during sleep in SAS patients to normal healthy volunteers and looked at the influence of apnea related arousals on CBFV. This study confirmed the results of Hajak et al 1994. While SAS patients had reduced CBFV levels than normal volunteers, both groups, again, showed slight decreases of CBFV during deepening sleep stages in the first sleep cycle. These results confirmed a sleep-stage-independent "bell curve" of CBFV with the high CBFV values upon sleep onset stage II and low values following

slow wave sleep. The authors suggested that this pattern may reflect a "sleep related circadian pattern of brain perfusion."

The impetus for the current study was based on the graphical depiction of the nighttime pattern of CBFV published in Hajak et al. (1994, 1996). This bell curve appears strikingly similar to the changes in human core body temperature during time free environment studies and during the constant routine protocol (Czeisler et al , 1985, 1986, 1989, 1990). The variation of CBFV across the sleep stages begins to look less like an "uncoupling of CBFV from cerebral electrical activity" and more like circadian variation. In the graphical depiction of core body temperature during a constant routine protocol, there is a precipitous decline in the first half of the night, a nadir usually mid morning, and a subsequent temperature rise at the start of the day. If one were to superimpose the pattern of CBT over the bell curve of CBFV found in Hajak et al. (1994, 1996) the two patterns appear to be similar.

4. EXPLANATION OF DEPENDENT VARIABLES
Physiological controls of CBFV

At least four main mechanisms regulate CBFV. The input variables are metabolic, external chemical, neurogenic, and pressure factors. The system can be divided in three main blocks: the dynamic pressure-flow relationship, the balance between supply and demand in the cerebral tissue, and the vascular smooth muscle regulating vasomotor tone (Radaideh et al 2002).

A. Metabolic regulation

CBFV is dictated by local cerebral metabolic activity. As demand increases, flow increases and vice versa. The actual coupling mechanism is unknown. A proposed control mechanism includes certain vasoactive compounds. Biochemical mediators include adenosine, potassium, and prostaglandins(Radaideh et al 2002).

B. External chemical regulation

1. Carbon dioxide (CO2)

PaCO2 is considered to be the most important known vascular modulator in CBFV. During periods of hypercapnia, the cerebrovascular smooth muscles relax. The mechanism of CO2 reactivity is regulated by extracellular H+. Systemic CO2 readily crosses the blood brain barrier which modulates extracellular H+ and then produces a change in the vascular smooth muscle properties. Critical components of the extracellular acid-base equilibrium are extracellular HCO3- and PCO2. The blood brain barrier is impermeable to bicarbonate and H+, but is freely permeable to gas. Molecular CO2

diffuses across the barrier and increases local PCO2 of the vascular muscle, reducing extracellular fluid pH and producing vascular relaxation. There is a 6% increase in CBFV per mm Hg change in PaCO2 when compared to normocapnic values (Hurn and Traystman, 2002). In the presence of hypercapnia, CBFV initially rises quickly, usually within 5 to 15 minutes. CBFV levels usually then return to baseline values, despite increasing levels of CO2 (Hurn and Traystman, 2002).

2. Oxygen

Reduced oxygen levels (hypoxia) produces increases in pial vessel diameter leading to marked vasodilation and increased CBFV (Radaideh, et al.2002; Hurn and Traystman, 2002). One brain region involved in neurogenic regulation of hypoxemic vasodilation is the rostral ventrolateral medulla (RVLM). Neurons in this area act as oxygen sensors. Hydrogen ions, adenosine, prostanoids, excitatory amino acids and other neurotransmitters also contribute to the vascular response to hypoxemia (Hurn and Traystman, 2002). An increased amount of oxygen (hyperoxia) produces mild vasoconstriction.

C. Pressure regulation

Cerebral perfusion pressure (CPP) autoregulation, i.e. the static flow-pressure relationship, is the driving force for blood through the cerebral circulation. It is defined as the difference between mean arterial pressure (MABP) and venous backpressure or intracranial pressure. Cerebral resistance arteries dilate during decreases and constrict during increases in mean arterial pressure and thus maintain relatively constant levels of

cerebral blood flow over a fairly broad range of cerebral arterial pressures. In normal adults, CPP is maintained over a gradient of MABP in the range of 60 to 160 mmHg. When CPP is reduced, CBFV is initially maintained by autoregulation and there is vasodilation of resistance arterioles. If CPP is further reduced, autoregulation capacity can no longer regulate CBFV and CBFV falls. When it falls, oxygen extraction fraction (OEF) maintains cerebral oxygen metabolism and tissue function only up to a point. Then, further CPP reductions lead to the point when reductions in OEF can no longer meet metabolic energy demands and ischemia will result (Radaideh, et al.2002).

CBF at any time can be described by the following formula:

CBF=(MAPB-ICP)/CVR=CPP/CVR

MAPB=Mean Arterial Blood Pressure

ICP=Intracranial Pressure

CPP=Cerebral Perfusion Pressure

CVR=Cerebrovascular resistance

D. Neurogenic regulation

Neural innervation of cerebral circulation can be divided into intrinsic and extrinsic systems.

1. The intrinsic system

This system includes the following brain structures: the medulla, including the dorsal medullary reticular formation and the rostroventrolateral medulla, the pons, including the locus ceruleus and the medial parabrachial nucleus, the midbrain including the dorsal raphe nucleus, the cerebellum, including the fastigial nuceus, and the forebrain, including the basal forebrain and the centromedian parafascicular thalamus. All of the aforementioned structures are involved in increasing cerebral blood flow velocity when stimulated, except for the areas of the pons. Stimulation of the locus ceruleus and parabracihal nucleus have demonstrated reductions in CBFV (Goadsby and Edvinsson 2002).

2. The extrinsic system

The extrinsic neural system includes the sympathetic nervous system and the parasympathetic nervous system. In the sympathetic nervous system, norepinephrine containing nerves arise from the superior cervical ganglion, course along the internal carotid artery, and innervate the large cerebral vessels. Neuroepinephrine is released and the arteries constrict. Parasympathetic innervation is a neural vasodilator. This system arises from the superior salivatory nucleus and passes out of the brain in the seventh cranial nerve (the facial nerve) fibers through pteygopalatine and otic ganglia to dilate the vessels. Acetylcholine, VIP, peptide histidine isoleucine, pituitary adenylate cyclase activating polypeptide, helodermin, helospectin I and II, and NO are all involved in the parasympathetic innervation (Goadsby and Edvinsson 2002).

Transcranial Doppler (TCD)

The current study utilizes Transcranial Doppler (TCD) ultrasonography to measure CBFV. It is a non-invasive instrument (consisting of one or two 2-Mhz transducers fitted o a headband) that is used predominantly as a diagnostic tool to assess cerebral hemodynamics in normal and pathological conditions. To measure CBFV, the instrument is typically placed on the head at one of three "windows" where intracranial arteries can be easily accessed through thin bone or natural foramina. The three windows, transtemporal, transforaminal (or suboccipital) and transorbital windows access different arteries. This study utilized the transtemporal window to access the middle cerebral artery (MCA). This is the most commonly used window because of its ability to directly access the middle cerebral artery (MCA) and posterior cerebral artery (PCA). The MCA is the artery from which CBFV was collected in this experiment. Transcranial Doppler is predicated on a theory that involves the measurement of moving objects when combined with radar. When the sound wave is emitted by the instrument, it is reflected by the blood cells that are in the path of the sound wave. The TCD in particular has been shown to detect changes in CBFV as a result of increases in end-expiratory PCO2 (Markwalder et al 1984; Ringelstein et al 1988).

Blood pressure

It is well known that blood pressure demonstrates a circadian rhythm with an increase in the morning and a decrease during sleep. Whether this rhythmicity is due to exogenous factors or endogenous factors has been the subject of great debate. The first few studies that have attempted to separate the external factors (i.e. activity) from

endogenous (modulated by circadian rhythms) evaluated blood pressure in immobile patients confined to 24-hour bed rest (Athanassiadis et al 1969; Mann et al 1979; Vand en Meiracker et al 1988; Van de Borne 1994). The overall results showed a reduced circadian rhythmicity of blood pressure in these patients suggesting that exogenous factors were playing a more significant role in blood pressure variability. Further studies suggested that other factors such as changes in behavior (i.e. sleep, activity), environment (i.e. caffeine), and activity (posture, exercise, talking) led to the rise in blood pressure in the morning (Baumgart et al 1991; Pickering and James 1993; Coca, 1994; Fukudome et al 1996; Kazuomi et al 1999).

The first study to systematically separate the endogenous from the exogenous rhythm of blood pressure used a constant routine protocol. With constant environmental conditions (including evenly distributed meals across the 24 hours and without the effects of sleep) there was no endogenous circadian variation of blood pressure (Kerkof et al 1998). This finding was replicated by Van Dongen et al 2001. The authors repeated the constant routine protocol five times in each subject and found the consistent absence of an endogenous variation in blood pressure.

Circadian markers

This constant routine protocol was first described by Mills et al 1978. The protocol is designed specifically to unmask underlying circadian rhythms in constant conditions. To assess for circadian phase the two major output markers of circadian rhythmicity, namely core body temperature and salivary melatonin (dim light melatonin onset) were collected. Additionally, blood pressure and end-tidal CO2 were collected because of their relationship to CBFV and cerebral hemodynamics.

A.Core body temperature

The core body temperature (CBT) and melatonin and rhythms are two of the most robust markers of the endogenous circadian timing system. There is a daily cycle in core body temperature that is independent of arousal states, sleeping and waking. The rhythm of CBT has a strong influence on the timing and duration of sleeping and waking. Under constant routine conditions, a 24 hour rhythm of CBT has been demonstrated on the constant routine protocol (Minors and Waterhouse, 1984 and Czeisler et al, 1985, 1986, 1989, 1990, 1992. The fitted minimum of core body temperature occurs an average of 1.4$\pm$ 0.3 hours before habitual wake time in healthy young men (Czeisler et al. 1992). Brown et al (1997) found that the onset of melatonin secretion occurs approximately 7 hours before the endogenous circadian temperature nadir. The offset was shown to occur approximately 40 minutes after the temperature nadir.

B. Melatonin

The SCN, located in hypothalamus, has a tight neuroanatomical link to the pineal gland in the brain. The pineal gland secretes the hormone melatonin into the body. The SCN governs the timing and production of melatonin (Watts 1991). The rhythm of melatonin can be shifted by light in humans through the neural links between the retinohypothalamic tract and the SCN. The effects of light on the melatonin rhythm have been well documented (for review see Shanahan et al 1997).

This study will also address the question of whether or not CBFV is a homeostatic process, governed by time spent awake, or a circadian process, governed by the circadian pacemaker. If CBFV is a homeostatic process, results should show a continuous decline

across the sleep deprivation period. If CBFV is a circadian process, it should track core body temperature, the major circadian output measure.

II. THE CURRENT STUDY

Previous studies have been limited by several factors. First, the environmental conditions (light level) and the behavior of the subject (sleep, meals, and caffeine intake) were not controlled. Second, CBFV measurements have occurred at only a few circadian phases. For example, Ameriso et al. 1994; Qureshi et al. 1999 assessed CBFV between 6-8 am, 1-3 pm, and 7-9 pm. Diamant et al 2002 assessed CBFV during the first 15 minutes of every hour and between 5-6:30 pm and 9:30-11:00pm. Given these brief time periods, it is impossible to obtain the true 24-hour profile. Third, primary output markers of the endogenous circadian pacemaker (such as core body temperature and melatonin production) were not assessed.

Materials and Methods

Subject Selection

Ten subjects (8 men 2 women; ages 24-33, mean 28 years; see Table 1) agreed to participate. One subject discontinued her participation because of a headache 15 hours into the study. Subjects were in good health, as assessed by medical history, semi-structured clinical interview, physical exam, and a cerebral vascular assessment by a trained Transcranial Doppler (TCD) technician. They reported no symptoms of sleep problems (such as insomnia, obstructive sleep apnea, narcolepsy, or restless legs syndrome).

Study Admission

Subjects that were selected to participate kept to a designated sleep-wake schedule (that was negotiated from the subject's typical pattern) and filled out a sleep diary for the two weeks prior to the time in the laboratory. Subjects tapered and discontinued their alcohol and caffeine intake so that for the entire week before the lab study they were not consuming alcohol or caffeine. During the data collection, subjects were not permitted either alcohol or caffeine.

Transcranial Doppler ultrasound recordings

The current study utilizes Transcranial Doppler (TCD) ultrasonography to measure cerebral blood flow velocity. It is a non-invasive instrument (consisting of one or two 2-Mhz transducers fitted on a headband) that is used predominantly as a diagnostic tool to assess cerebral hemodynamics in normal and pathological conditions. To measure CBFV, the instrument is typically placed on the head at one of three "windows" where

intracranial arteries can be easily accessed through thin bone or natural foramina. The three windows, transtemporal, transforaminal (or suboccipital) and transorbital windows access different arteries. This study utilized the transtemporal window to access the middle cerebral artery (MCA). This is the most commonly used window because of its ability to directly access the middle cerebral artery (MCA) and posterior cerebral artery (PCA). The MCA is the artery from which CBFV was collected in this experiment. Transcranial Doppler is predicated on a theory that involves the measurement of moving objects when combined with radar. When the instrument emits the sound wave, it is reflected by the blood cells that are moving in the vector of the sound wave (Aaslid 1992).

CBFV was measured using either the right or left middle cerebral artery (MCA) using Transcranial Doppler sonography (TCD: DWL Multidop X-2, DWL Elektronische Systeme GmbH, D-78354 Sipplingen/Germany) through the temporal window. To obtain an optimal signal, the TCD probe was first covered with an ultrasound conducting gel. The position of the probe was then adjusted on the temple until audio signal and a clear waveform appeared on the TCD monitoring screen. The depth of the range-gate was then increased or decreased in a stepwise manner until the optimal signal to noise ratio was obtained. When the 2MHz stimulation identified the MCA (typically at a depth of about 55 mm) the TCD probe was fixed to the head with a plastic head frame (MARC500, Spencer Technologies, Nicolet Biomedical Inc.). An observer who was present continuously during the recordings evaluated the quality of the signal. This enabled long-term recording of CBFV throughout the study. Fast Fourier transformation (FFT) of the signal was used to analyze the velocity spectra. The mean velocity of the middle cerebral

artery (MCAV) was obtained from the integral of the maximal TCD frequency shifts over one beat divided by the corresponding beat interval and expressed in cm/s. Analysis was conducted off line.

Laboratory Constant Routine Protocol

The study protocol was approved by the Institutional Review Boards of New York Presbyterian Hospital – Weill Medical College of Cornell University and The City College of New York. Subjects gave written and informed consent before participating. Subjects arrived at the laboratory between 9:30 am and 10:00 am. Electrodes were placed on the subject's head and face as they sat in a chair next to the bed. Light levels were below 150 lux. Sleep logs were collected. The plastic head frame of the TCD was fitted to the subject. A manual TCD probe was prepared with conducting gel and placed on either the right or left side of the subject's head. Optimal signal was identified visually on the TCD monitoring screen and by an audible signal. Once a signal was identified by sound, the depth of the signal was adjusted until there was a clear visual representation of the waveform on the monitor.

Data collection began at 11 am. Subjects remained in bed and awake in a semi recumbent position for 30 hours in an established "constant routine" (CR) protocol. Subjects remained in low (<25 lux) light levels so that it had little or no entraining effect on the circadian pacemaker (Boivin et al 1996). They were not allowed to get out of bed to urinate. Instead they urinated in private in a urinal or bedpan. Subjects remained awake from 11:00 a.m. on Day 1 until 5 p.m. on Day 2. Throughout the study, subjects were provided small meals (Ensure liquid shake plus one-quarter nutritional food bar) every 2 hours. Subject's typical total food and liquid intake for a day and a quarter were

divided into 15 relatively equal portions. This protocol represents a modified CR as subjects were allowed television and were therefore were not in "time isolation." Additionally, subjects needing to defecate were allowed out of bed to go to the bathroom, which was located a few steps away from the bedside.

Measurement of standard markers of the circadian pacemaker

Body temperature recordings

Core body temperature was recorded at 1-minute intervals with an indwelling rectal probe (MiniMitter, Co. Bend, OR). A wire lead connected the sensor out of the rectum to a data collection system worn on the belt. Temperature readings were collected and saved into the device. The readings could be viewed by connecting a lap –top computer to the device. The investigator monitored the temperature on the lab top computer approximately every hour of each of the constant routines, save for approximately 4-5 hours total. During the 4-5 hour time periods in which she was not available, another investigator was responsible for monitoring the CBT. After the study the recordings were visually inspected and artifacts resulting from removal or malfunction of the probe were excluded from further analysis.

Salivary Melatonin

Salivary samples of 3 ml were collected every hour from 11:00 a.m. on Day 1 to 4:00 p.m. on Day 2. Ten of these samples were used for the determination of the timing of the dim light melatonin onset (DLMO) and peak melatonin levels. Subjects were asked to rinse their mouths with water 15 minutes before the saliva sample was produced. Subjects were then provided with a plastic conical tube and asked to expectorate 3ml of saliva. For each subject, DLMO was assessed across a ten-hour time window. DLMO

was assessed across a time window that included the ten hours prior to the subject's core body temperature minimum (CBT minimum). Immediately after collection, each saliva sample was frozen and stored at -20°C. Saliva samples were assayed using Bühlmann Melatonin Radio Immunoassay (RIA) test kit for direct melatonin in human saliva (American Laboratory Products Co., Windham, NH). Analysis was conducted at New York State Institute for Basic Research. Dim light melatonin onset time was selected in each subject based on two criteria. A saliva sample needed to have melatonin concentration 3 pg/ml or above and later samples needed to show higher levels (Bühlmann laboratories). Second, 3 pg/ml threshold needed to occur within 6-10 hours before core body temperature minimum (Brown et al. 1997; Lewy, 2003 personal communication).

Polygraphic recordings

Electroencephalography (EEG) was continually assessed across the 30 hours to ensure that subjects maintained wakefulness. The following montage was used according to the international 10-20 system: C3-A2, C4-A1, O1-A2, O2-A1, ROC-A1, LOC-A2, and submentalis EMG. One channel of electrocardiogram was continuously recorded by monitoring from two electrodes (one on each side of the body at the shoulder chest junction). The EEG software (Rembrant Sleep Collection Software Version 7.0) was used for data acquisition and display of the signals on a personal computer. Throughout the CR, the investigator monitored the quality of the recordings. The recordings were scored by the principle investigator.

Blood pressure recordings and systemic hemodynamic variables

An automated blood pressure cuff was placed on the bicep of the subject and inflated two times each hour in order to determine changes in blood pressure over time. Blood pressure in one subject (02) was recorded via a finger blood pressure monitor (Omron Marshall Products, Model F-88). Blood pressure in subjects 03, 04, 05, 06 and 13.5 hours of 07 were recorded with Omron Healthcare, Inc, Vernon Hills, Illinois 60061 Model # HEM-705CP Rating: DC 6V 4W Serial No: 2301182L. BP for the remaining 16.5 hours of subject 07, and the entire 30 hours of subjects 08, 09 and 10 were recorded with a similar blood pressure monitor (CVS Pharmacy Inc, Woonsocket, RI 02895 Model # 1086CVS Measuring range = 20 - 280 mmHg measuring intervals = 1 mmHg Rating DC6V 3.5W).

End-tidal carbon dioxide

CO2 was continuously obtained. A nasal cannula for monitoring expired gases was placed under the nose. Relative changes in carbon dioxide content were measured by an Ohmeda 4700 Oxicap (BOC healthcare). Mean CO2 levels were analyzed off-line. As part of a separate study, twice per hour, subjects completed a focus procedure during which they were asked to focus on an object for three minutes. During this period they were asked to avoid blinking and body movement at this time. The mean of the peak CO2 values were obtained during this three-minute period because subjects kept their head still and looked straight ahead, which ensured that movement artifact would not affect the CO2 reading. Offline analysis of the CO2 values on the PSG revealed a sinusoidal CO2 waveform. The waveform had several values ascribed to various points

along the wave. We chose to record (by hand) the value at the peak of the sinusoidal wave. The peak values were recorded and averaged across the three minute time period (6 epochs) every half hour. We chose the mean peak CO2 for two reasons. First, it was the most distinct point on the CO2 sinusoidal wave and secondly, it was a convenient sampling method.

Data Analyses

Data reduction and Statistical Procedures

CBT values were first subject to data rejection. All CBT values that were less than five standard deviations below the subject's respective mean were rejected. CBFV values were also subject to data rejection. All values less than 20 cm/s were determined to be artifact according to Alan Segal, MD, personal communication. CBFV values below 20 cm/sec were chosen to be artifact because they are so slow they may indicate a severely abnormal vessel, such as in the setting of stroke. Given that we were looking at healthy volunteers, with no neurological symptoms, such values were much more likely artifact (or the probe not being optimally centered on the vessel). Secondly, sometimes with TCD, one may insonate a vein rather than artery; 20cm/sec is a much more appropriate value for a vein than an artery and one could therefore conclude that actually looking at a vein rather than an artery. Data reduction was accomplished by averaging into one minute, 30 minute or hourly bins. To ensure that circadian measurements were made under basal conditions, the first five hours of the constant routine was excluded from all analyses to eliminate effects of study adaptation. The last hour was excluded to eliminate confounding expectation effects. Data is presented in this article in three ways. First, CBT and CBFV values were according to time of day. Second, CBFV values were

aligned according to the temperature nadir and third, the CBFV nadir was aligned to the CBT nadir. To align CBFV to the CBT circadian nadir, the CBT nadir of each individual subject was set to circadian time 0, or 0°. The CBFV value that corresponded to the CBT nadir was then also set to 0. Each half hour data point after the temperature nadir and corresponding CBFV values were then set to a circadian degree. There were a total of 48 data points across the 24 hour period. Therefore, each data point was equal to 7.5 degrees so that each data point would accumulate to 360°. Lastly, mean values were obtained for CBT and CBFV at each circadian degree. To align both the CBFV nadir to the CBT nadir, first, the lowest value of CBT and the lowest value of CBFV was identified and set to circadian time 0, or 0°. Subsequent data points were aligned as just described. To determine the phase difference between the CBT nadir and the CBFV nadir, first, the time at which the CBT nadir occurred was identified in individual subject's data. Second, the time at which the CBFV nadir occurred was identified. The difference between these times was then calculated and averaged.

Estimation of circadian phase

To identify circadian components of the CBT and CBFV data, first, power densities were obtained using FFT of the CBFV and CBT values for each subject conducted (SAS version 8.2-proc spectra program for pc, SAS Institute, Cary, NC). Data was smoothed using a 7 point symetric triangular window with weight of 1, 2, 3, 4, 3, 2, 1 for the three points preceding the current point, the current point and the three following the current point, respectively. Then, autocorrelations on CBT and CBFV values were run displaying the data on a periodogram to determine whether a cycle was present in the data. All values were then mapped to a sine wave using a fast fourier transform (FFT)

and displayed on a spectral density graph. The spectral density graph was then examined visually to determine the size of the cycle for the CBT and CBFV in each subject. The presence of cycles in CBT and CBFV were then tested for "white noise" with Fisher's Kappa and Bartlett's Kolmogorov-Smirnov statistics.

Second, CBT and CBFV curves were fitted with a 24 hour nonlinear least squares regression procedure (SAS Institute, Cary, NC). This technique constrains the circadian period of CBT and CBFV to be within 24 hours. This technique used the following equations: model cbt=&avg_cbt+r*cos((2*3.1415)*(hours-&max_cbt)/24; model cbfv=&avg_cbt+r*cos((2*3.1415)*(hours-&max_cbfv)/24 (where &=constants that center the curve at the actual average for each series (vertical centering) and the predicted maximum at the actual maximum (horizontal centering); r=the amplitude of the cosine wave. In addition, the regression analysis also yielded an estimated clock time for the CBT nadir and CBFV nadir (Synergy software, Kaleidagraph Version 3.6). Third, the minimum of the circadian rhythm of CBT and DLMO were also used as markers of the endogenous circadian phase.

Results

The TCD probe was placed on either the right or left temple, whichever gave the better signal. Mean isonation depth was 56.5 mm for the right MCA and 55.6 mm for the left MCA (range 53-60 mm). For six of the nine subjects, the TCD probe was placed initially on the right side and for three of the nine subjects, on the left side. In two subjects, the probe was switched from the left side of the head to the right side because of discomfort with the placement of the probe. The constant routine ranged from 28-30

hours in duration. The first five hours and the final hour of data from the constant routine were excluded from analysis.

Circadian phase position

Phase position was determined for the rhythms of CBT and CBFV. The overall mean (n=9) circadian phase position for CBT occurred at 4:00 am (Figure 1) and CBFV occurred at 9:30 am (Figure 11). There was no significant difference between the mean circadian phase of CBT and CBFV (t=-0.52, DF=8, p=0.62). Individual subject data are shown below.

Core body temperature

A 24 hour non-linear multiple regression, cosine fit analysis revealed that the overall mean CBT rhythm (n=9) fit a 24 hour cosine rhythm (R^2=.79), Figure 1. Cosine curve fits for CBT in individual subjects are displayed in Figures 2 through 11. With a fast Fourier transform (FFT) it was possible to detect whether there were periodicities of different wavelengths in the CBT data. Cycle length was identified visually from spectral density graphs. A cycle length of approximately 24 hours was detected in CBT data in all nine subjects (p= <0 .01). Salivary dim light melatonin onset (DLMO) also served as a measure of endogenous circadian phase position.

Cerebral blood flow velocity

A 24 hour- non-linear multiple regression, cosine fit analysis revealed that the CBFV rhythm fit a 24 hour cosine rhythm (R^2=.50), Figure 11. Figures 12 through 20 display the CBFV for individual subject data across the 24 hour period. FFT analysis revealed that seven out of nine subjects showed an approximately 24 hour cycle in CBFV. The other two subjects (Subject 08 and Subject 09) showed shorter and

sometimes multiple cycles in CBFV of 12, 4 and 8 hours. Although these two subjects did not show a 24 hour rhythm of CBFV, we felt the most appropriate way to present the data was to include these subjects in the overall analysis.

The relationship between CBFV and CBT

CBFV was analyzed according to its relationship to the circadian phase of CBT. The relationship between CBT and CBFV is presented here in three ways. First, the pattern of CBT and CBFV values are presented according to time of day (Figure 21). Second, the two variables are present with respect to CBT nadir (Figure 22). For each individual's data, the CBT nadir was designated to circadian time 0. The corresponding CBFV values were aligned accordingly and averaged across subjects (see methods section). This resulted in averaging across subject's CBFV at the same circadian phase. Third, both CBT and CBFV are aligned according to their respective nadirs (Figure 23). All figures are double plotted.

CBFV rhythm is about 90 degrees out of phase from the CBT rhythm

The average difference between CBT nadir and CBFV nadir was 6 hours or 90 degrees. In individual subject data, the differences ranged from 1 hour to 9.5 hours. Seven out of nine subjects had differences of CBFV in the negative direction (CBFV rhythm was shifted to an earlier time in order to align to CBT nadir). Two subjects (03 and 08) had positive differences (CBFV rhythm was shifted to a later time in order to align to CBT nadir). The average phase difference between CBT acrophase and CBFV acrophase was 8 hours or 120 degrees. In individual subject data, the differences ranged from 1 to 15 hours. All differences were in the negative direction. The average phase

difference of the CBT midpoint and CBFV midpoint was 6 hours or 90 degrees. The individual subject data revealed differences that ranged from 0 hours to 8 hours. Five subjects (05, 06, 07, 09, and 10) had positive differences (shifted to a later time) and three subjects (02, 03, and 08) had negative differences. In one subject, the midpoint of the two parameters occurred at the same time (zero shift). The difference between the mean circadian phase position according to the cosine curve of CBT and CBFV was 5.5 hours.

Data were then separated according to bins of 60 degrees (equal to four hour segments) across the constant routine. In Tables 3 and 4, columns two through seven show the correlation between CBT and CBFV for each 60-degree temperature phase. The correlations for the first 180 degrees of the study and the second 180 degrees of the study are in columns eight and nine, respectively. Column 10 shows the overall correlation across the circadian cycle. Correlations in columns two through seven include 18 data points, columns eight and nine 50 data points, and column 10 includes 98 data points. When the phase of CBFV was shifted so that the lowest value was aligned to the lowest CBT value, the two parameters were highly correlated (see Figure 2; $r=0.76$, $n = 98$ $p=<.01$). Table 4 shows that between 180-240 degrees, both CBT and CBFV decline ($r=0.71$, $p=<01$) and again between 300 and 360 degrees, just before CBT nadir ($r=0.85$, $p=<.01$). CBFV and CBT declined together across the first half of the circadian period (0°-180°) ($r=0.64$, $p=<.01$) and rose together in the second half (180°-360°) of the circadian period ($p=0.88$, $p<01$). A 24 hour- non-linear multiple regression, cosine fit analysis revealed that when both the CBT and CBFV rhythms are aligned to their respective nadir, the non-linear 24 -hour cosine fits were $R^2=0.70$ and $R^2=0.68$, respectively.

When aligned to their respective nadirs, the mean CBT was 98.6 F (SD = 0.24) and the mean CBFV was 39 cm/s (SD = 3.53) Overall, Fisher's z-transformed values revealed that the amplitudes of the two parameters were similar while the difference in their variability was large. The amplitude of CBFV was a z score of 5.75 and CBT was a z score of 3.96. Table 5 shows a positive correlation between CBT and pulse rate ($r=0.40$, $p<.01$). Diastolic blood pressure and CBT had a negative correlation ($r=-0.29$, $p<.01$). CBFV and CO2 were positively related ($r=0.24$, $p<.05$) which is consistent with (Markwalder et al 1984; Ringelstein et al 1988; Aaslid et al 1989) showing that changes in CO2 are associated with changes in CBFV. CBT and pulse were also significantly correlated ($r=.40$, $p<.01$).

Discussion

This study is the first to use the constant routine (CR) protocol to determine whether the endogenous circadian pacemaker contributes to the previously reported diurnal changes in CBFV. The current work demonstrates that, with limited periodic external stimuli and a constant posture, a cycle length of approximately 24 hours in CBFV was apparent. Subjects showed a cycle of approximately 24 hours in CBT, which has been previously demonstrated with the CR (Czeisler et al, 1980;Czeisler et al 1992;Czeisler et al 1999). Aligning both rhythms revealed a 90 degree phase angle difference between CBT and CBFV with CBFV delayed in relation to CBT. Aligning each of these variables to their respective nadirs and then aligning the nadirs resulted in CBFV closely tracking CBT as demonstrated by the substantial correlation between these two measures. The phase angle difference between the CBT and CBFV rhythms may

help explain alertness in the evening before habitual bedtime and what has been called sleep inertia in the morning after waking up.

At the time around the CBT acrophase the relationship between the two rhythms undergoes a transition. Between 180 and 240 degrees, CBFV is still rising and CBT is changing directions (first rising, reaching its peak and then falling). This period between 180 and 240 has been described as a "wake maintenance zone" a time in the circadian cycle during which humans are less likely to fall asleep (Strogatz et al 1987). In our subjects, the CBT is near its zenith or just starting to fall at this time and CBFV is still steadily rising. Higher values in CBT and CBFV are associated with activation and therefore these two endogenous rhythms may be promoting wakefulness during this "wake maintenance zone". Between 240 and 300 degrees, CBVF is still rising, CBT is steadily declining, and the correlation between the two variables is negative, but not quite significant ($r = -0.60$, $p=0.09$). This continuing rise in CBFV may be an indicator of how people function well and stay awake before bedtime when the homeostatic drive for sleep is approaching its highest levels and the endogenous circadian alerting influence, as indexed by CBT, is rapidly falling. The still rising CBFV values at this period around habitual bedtime may reflect the continued activation of the cerebral cortex, which may facilitate waking at a time when the two process model (Borbély, 1982;Daan et al 1984) predicts increased tendency for sleep. As wakefulness was extended past the subject's habitual bedtime, both rhythms decline together ($r=.85$, $p<.01$). Between 0 and 60 degrees CBFV steadily declines and CBT is steadily rising (CBFV:CBT, $r = -0.56$, $p = .12$). In the morning, the phase delay of the CBFV rhythm in relation to CBT may

contribute to grogginess, performance impairments (Jewett et al 1999), or what has been called sleep inertia.

In the few hours before the study's termination, (at approximately 140 degrees see Figure 21) there was a spike in both CBFV and CBT and may have been a result of this modified constant routine. In a traditional constant routine protocol, subjects remain in time isolation. However, subjects in this study were allowed to watch television and listen to the radio. Therefore, they were aware of the current time as well as when they were approaching the conclusion of the study. The increased arousal produced by knowledge that the study is about to end may have accounted for this spike.

Previous studies using simultaneous EEG and TCD to continuously measure CBFV across the sleep period have concluded that, except for periods of REM sleep, (Madsen et al 1991a, 1991b), there is a linear decline in CBFV across the night. At first this was attributed to different stages of sleep (Townsend et al 1973; Sakai et al 1979; Sakai 1980; Sakai et al 1990; Meyer 1981; Gozukirmizi 1982 ;Meyer et al 1987;Madsen et al 1991;Heiss et al 1985; Maquet et al 1988; Buchsbaum et al 1989;Maquet et al 1990; Madsen et al 1991;Klingelhoeffer et al 1992; Droste et al 1993;Hajak et al 1994; Hajak et al 1996; Kuboyama et al 1997; Meadows et al 2003). In later studies, the decline in CBFV through the night was interpreted as a "decoupling" of cerebral electrical activity and cerebral perfusion during non-REM sleep (Klinghelhoeffer et al 1992; Hajak et al 1994; Hajak et al 1996). In all studies (Klinglehoffer 1992, Droste 1993;Hajak et al 1994, Hajak 1996; Kuboyama 1997) CBFV values were lower in the morning during wakefulness than during wakefulness prior to sleep at night. The current findings show that the decline in CBFV is present during wakefulness in the nighttime hours and

therefore cannot be attributed to sleep and associated changes (including factors such as the shift to recumbency, and reduced activity, metabolic rate and respiratory rate).

Our results demonstrate that the CBFV nadir occurs about six hours (90 degrees) after the CBT nadir. The phase angle difference between CBT and CBFV may be secondary to the sleep deprivation that is part of the constant routine. Brain imaging studies across sustained periods of wakefulness have shown significant decreases in absolute regional cerebral glucose metabolic rate in several areas of the brain. These areas include the thalamus and the cerebellum after 32 hours of sleep deprivation (Wu et al 1991), the prefrontal anterior cingulated gyrus, the lateral posterior patrietal lobules, the pulvinal thalamus, and the visual cortices after 35 hours of sleep deprivation (Drummond et al 1999). In addition, prolonged wakefulness is associated with decreases in metabolic rate in the thalamus, prefrontal, and posterior parietal cortices after 24 hours of sleep deprivation (Thomas et al 2000), which are regions involved in alertness and attention. To our knowledge, other studies have only looked at the effects of total (Volk et al 1992; Gillin et al 1992) and partial (Volk et al 1997) sleep deprivation on CBFV values and ratings of depression. These studies also showed CBFV values to be lower in the morning following the sleep deprivation than the night before. It is interesting to note that one subject in this study, (subject 03), reported sleeping approximately five hours the night before the study. Mean CBFV values in this subject were approximately 20 cm/s lower than the mean values of the other subjects

Covariates of CBFV: blood pressure, heart rate, and CO2
Blood pressure

Systolic blood pressure was not correlated with CBT and there was a small inverse relationship between diastolic blood pressure ($r=-0.29$, $p=<.01$) and CBT. This

is consistent with previous studies (Mann and Miller Craig 1979; Pickering et al 1982; Kerkoff et al 1988; Baumgart et al 1991; Pickering and James 1993; Coca 1994; Fukudome et al 1996; Kazuomi et al 1999; VanDongen 2001) suggesting that the rise in blood pressure in the morning is due more to external factors (i.e. sleep, exercise, talking, posture, and caffeine) than to endogenous circadian regulation.

CO2

Mean end-tidal CO2 values were significantly correlated with CBFV across the study period ($r=0.24$, $p=<.05$). This finding supports CO2's role as a major regulatory parameter for the control of CBFV. These results also suggest that CO2 regulation of CBFV is maintained despite total sleep deprivation. The relationship between CO2 and CBFV in sleep may be more variable. CBFV values in patients with sleep apnea have been shown decline across the night, despite increases in CO2 and blood pressure (Klingelhoeffer et al 1992). There was a small negative correlation between end- tidal CO2 and CBT ($r=-0.21$, $p<0.5$), which is consistent with the findings of Spengler et al. (2000) who showed a consistent but small amplitude circadian rhythm in mean end-tidal CO2 on a CR protocol. With respect to heart rate, there was a strong positive correlation with CBT ($r=0.40$ $p=<.01$). These results suggest that there may be circadian component to heart rate and is consistent with those of Van Dongen et al (2001) who also found a reliable circadian rhythmicity of heart rate on a CR protocol. There was no correlation between heart rate and CBFV ($r=-.08$, p=ns).

Melatonin

We found the average difference between the CBT nadir and dim light melatonin onset (DLMO) was 7.71 hours. This is consistent with Brown et al 1997, who showed

that the onset of melatonin secretion occurred an average of just over 7 hours before CBT nadir. Shanahan and Czeisler (1991) found that the fitted maximum of melatonin secretion occurred an average of 1.9+/- 0.3 hours prior to the fitted temperature minimum. We found that the difference between the maximum of melatonin secretion and CBT nadir was slightly longer, at 2.79 hours. The average difference between CBFV nadir and DLMO was longer, at 10 hours, and consistent with the overall delay in this rhythm (though not significant, p=0.62).

Clinical correlation

The approximate 6 hour (90 degree) phase angle difference between the CBFV and CBT is consistent with a time window in the morning during which several physiological changes have been observed. For example, cerebral vasomotor reactivity to hypocapnia, hypercapnia, and normoventilation has been found to be most reduced in the morning (Ameriso et al 1994; Qureshi et al 1999; Meadows et al 2003). In addition, Toffler et al (1987) found that across a 24 hour period, there was a significant morning (6 am to 9 am) increase in platelet aggregability. The low CBFV values in the morning may help explain the results of a meta-analyses which found a 49% increased risk of strokes in the morning compared to the afternoon or evening (Elliot, 2001; Tsementzis et al. 1985; Wroe et al 1992; Feigin et al 2001). The increased frequency of the incidence of stroke has been found in the different subtypes of strokes. For example, incidences of subarachnoid hemorrhage (SAH), intracerebral hemorrhage (IH), and thromboembolic hemorrhage (TH) are higher in both men and women (ages <65 and >65 in Feigin et al 2001 and over age 70 Tsementzis et al. in 1985) between the hours of 10:00 am and 12:00 pm. The lowest incidences of all types of strokes occurred between 12:00 a.m. and

6:00 a.m. The increased incidence of these events has been attributed, in part, to the surge of blood pressure in the morning when patients are getting out of bed (Feng et al 1999; Muller 1999a, 1999b; Diamant et al 2002).

Our results demonstrate that even in the absence of surges in blood pressure, the phase of CBFV reaches it's lowest values during the hours before 12 pm. This further suggests that the endogenous rhythm of CBFV may increase the risk of CVAs in the late morning hours even without changes in posture or activity. The decline in CBFV while lying quietly in bed may predispose vulnerable patients to the onset of MIs or CVAs.

Overall, these study results demonstrate that CBFV, in the absence of sleep, exhibits properties of a circadian rhythm as it rises and falls across a 24 hour period. We found a 90-degree phase angle difference, though not statistically significant, in the CBFV rhythm with respect to the CBT rhythm. This delay is consistent with the lower CBFV values found in the morning in previous studies and corresponds to the time period of during which there is an increased incidence of strokes. The mechanisms by which delay is occurring have yet to be explained.

Table 1.

Exclusion criteria were as follows:

- cerebrovascular disease such as stroke, stenosis or arteriosclerosis,
- recent head trauma.
- a seizure disorder
- current or past psychosis
- current unstable medical conditions
- current infection
- sleep disorders such as Insomnia, Narcolepsy, Obstructive Sleep Apnea and Restless Legs Syndrome
- taking medications for anxiety (benzodiazepines) depression (tricyclics, quadricyclics, specific serotonin reuptake inhibitors) and neurological problems (barbiturates, other sedating medications and anti-seizure medications)
- use of recreational drugs (e.g., marijuana, amphetamines and opiates) in the past month
- smoke
- drink more than 3 cups of coffee per day
- consumes more than 7 alcoholic beverages per week
- jet travel across time zones in the past 8 weeks

Table 2. Characteristics of subjects

Subject	AGE (YEARS)	SEX	SIDE MEASURED
02	29	Male	Right
03	28	Male	Right
04	30	Male	Right
05	33	Male	Left
06	26	Male	Left
07	27	Female	Right*
08	24	Male	Right
09	28	Male	Right*
10	26	Male	Left

Subject 09: TCD was switched from the left to the right side of the head seven hours into the study. Subject 07: TCD was switched from left to right two hours into the study.

Table 3 Correlations among cerebral blood flow velocity and core body temperature according to circadian phase

CIRCADIAN DEGREES

1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Degrees	0°-60°	60°-120°	120°-180°	180°-240°	240°-300°	300°-360°	0°-180°	180°-360°	OVER-ALL
Overall (r)	-0.56	0.34	-0.88**	0.05	-0.60	0.85**	-0.21	-0.32	-0.06
Sig.(2-tailed	0.12	0.38	0.00	0.90	0.09	0.00	0.30	0.13	0.70
N	9	9	9	9	9	9	25	25	49

*Significant at the 0.05 level (2-tailed)** Significant at the 0.01 level (2-tailed)

Table 4 Correlations among cerebral blood flow velocity and core body temperature when both variables are aligned to their respective nadir

	CIRCADIAN DEGREES								
1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Degrees	0°-60°	60°-120°	120°-180°	180°-240°	240°-300°	300°-360°	0°-180°	180°-360°	OVER-ALL
Overall (r)	0.35	-0.42	0.23	0.71**	0.58	0.85**	0.64**	0.88**	0.76**
Sig. (2 tailed)	0.34	0.91	0.56	.033	0.10	0.00	0.00	0.00	0.00
N	9	9	9	9	9	9	25	25	49

*Significant at the 0.05 level (2-tailed)** Significant at the 0.01 level (2-tailed)

Table 5. Blood pressure, heart rate, and end-tidal CO2 according to time of day

OVERALL (R)	SYS-TOLIC BP	DIAS-TOLIC BP	PULSE	CO2	CBT	CBFV
CBT	-0.05	-0.29**	0.40**	-0.21*	1.0	-0.06
CBFV	0.12	0.09	-0.08	0.24*	-0.06	1.0

*Significant at the 0.05 level (2-tailed)** Significant at the 0.01 level (2-tailed)

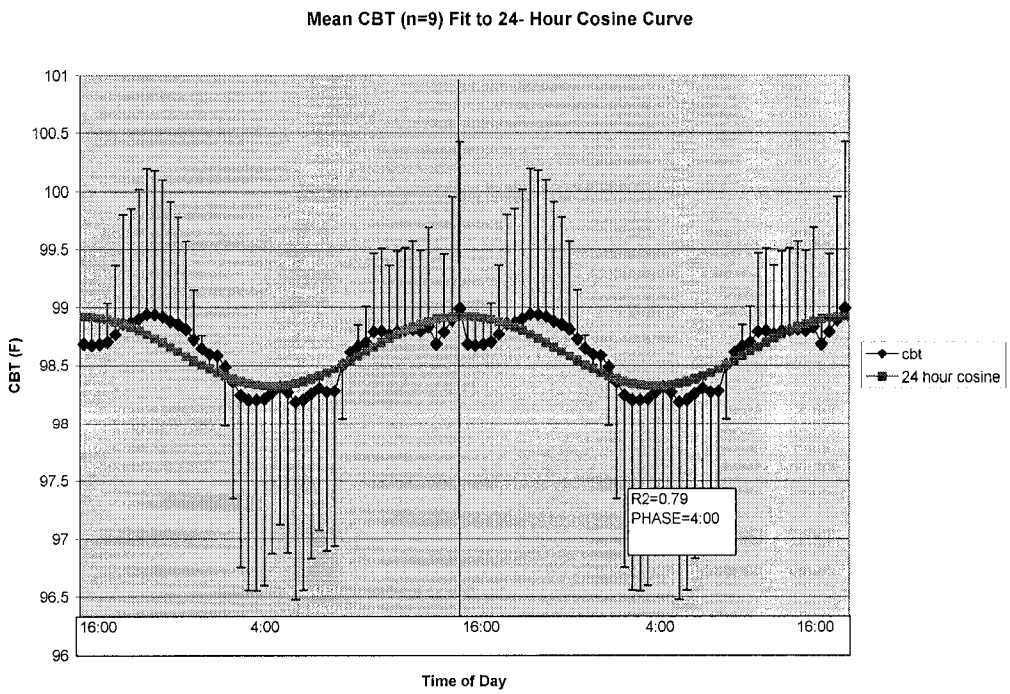

Figure 1. Time course of CBT across 9 subjects according to time of day. Shown is a double plot of the group (n=9) mean levels (+/- standard deviation) of CBT (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBT values (degrees F). The vertical line indicates where the data was folded. Also displayed in the lower right corner is the non-linear cosine curve fit for mean CBT, $R^2 = 0.79$. The overall mean circadian phase position was 4:00 am.

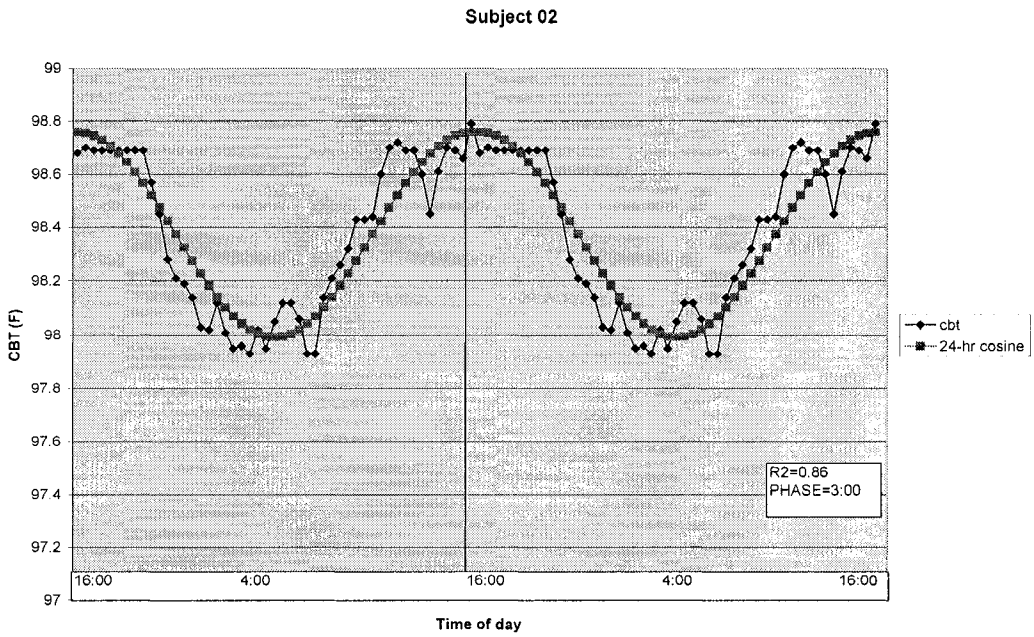

Figure 2. Time course of CBT in subject 02 according to time of day fit to a 24 hour cosine curve. Shown is a double plot of CBT (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBT values (degrees F). The vertical line indicates where the data was folded. Mean CBT = 98.4, SD=0.3. Also displayed in the lower right corner is the non-linear cosine curve fit for CBT in subject 02, $R^2 = 0.86$. The circadian phase position in this subject was 3:00 am..

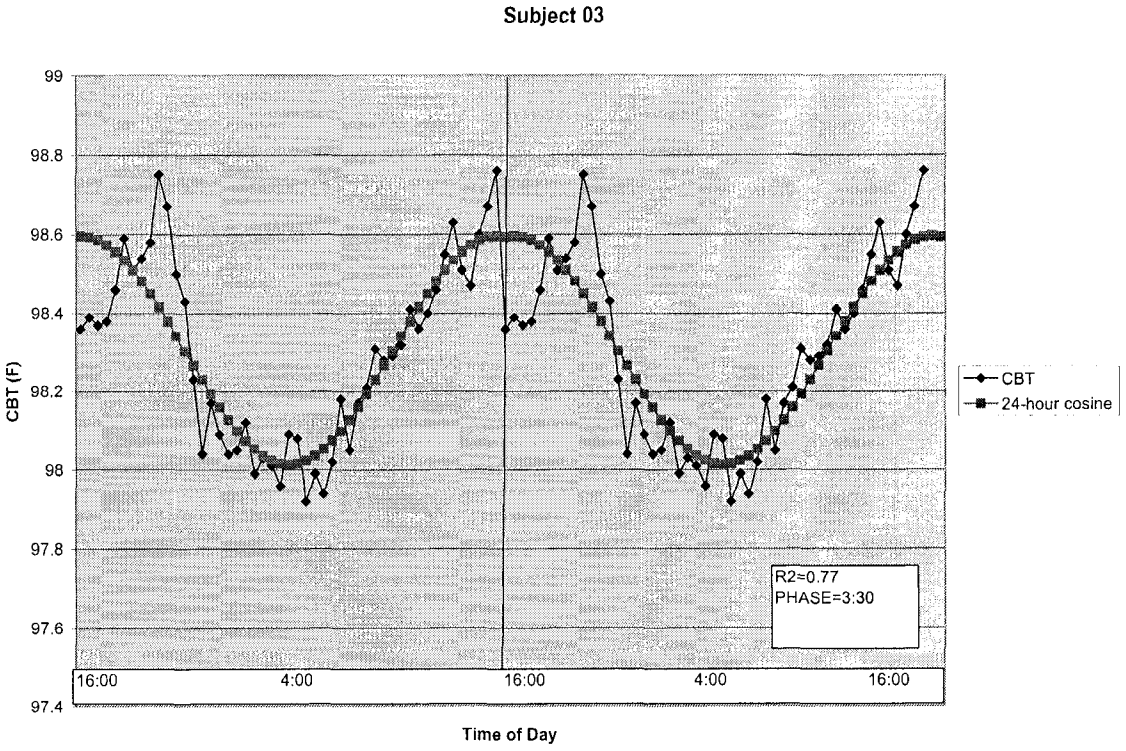

Figure 3. Time course of CBT in subject 03 according to time of day fit to a 24 hour cosine curve. Shown is a double plot of CBT (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBT values (degrees F). The vertical line indicates where the data was folded. Mean CBT=98.3, SD=0.2. Also displayed in the lower right corner is the non-linear cosine curve fit for CBT in subject 03, $R^2 = 0.77$. The circadian phase position in this subject was 3:30 am.

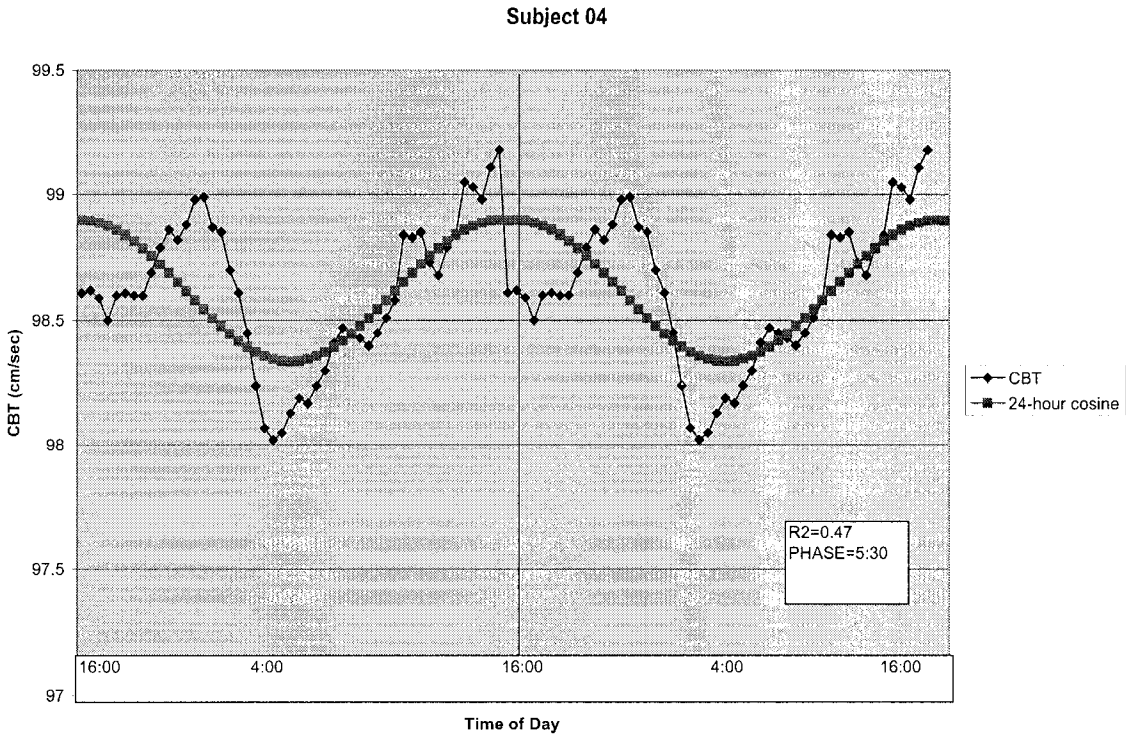

Figure 4. Time course of CBT in subject 04 according to time of day fit to a 24 hour cosine curve. Shown is a double plot of CBT (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBT values (degrees F). The vertical line indicates where the data was folded. Mean CBT=98.6, SD=0.3. Also displayed in the lower right corner is the non-linear cosine curve fit for CBT in subject 04, $R^2 = 0.47$. The circadian phase position in this subject was 5:30 am.

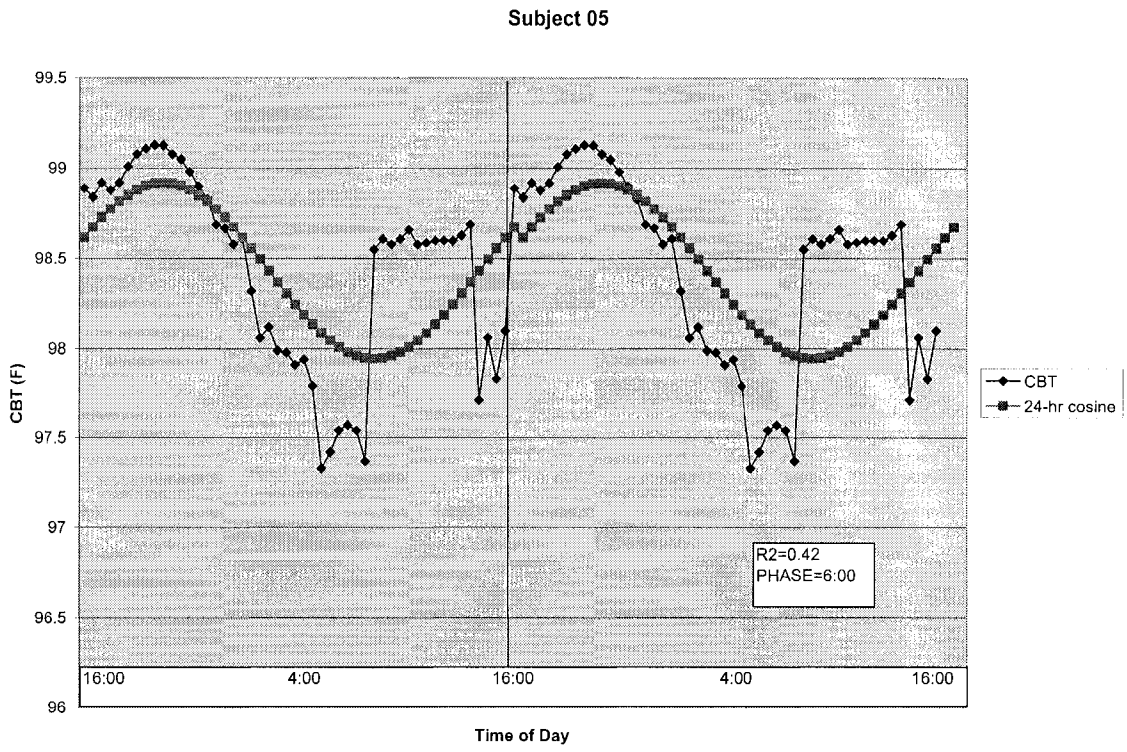

Figure 5. Time course of CBT in subject 05 according to time of day fit to a 24 hour cosine curve. Shown is a double plot of CBT (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBT values (degrees F). The vertical line indicates where the data was folded. Mean CBT=98.4, SD=0.5. Also displayed in the lower right corner is the non-linear cosine curve fit for CBT in subject 05, $R^2 = 0.42$. The circadian phase position in this subject was 6:00 am.

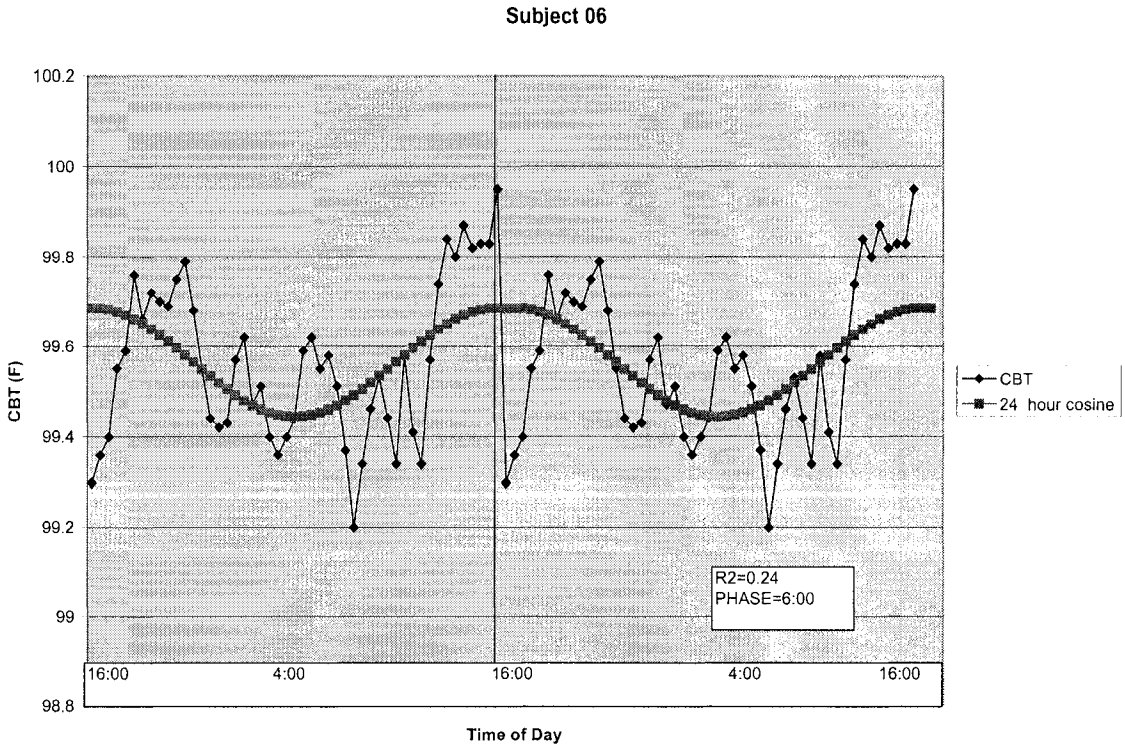

Figure 6. Time course of CBT in subject 06 according to time of day fit to a 24 hour cosine curve. Shown is a double plot of CBT (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBT values (degrees F). The vertical line indicates where the data was folded. Mean CBT=99.6, SD=0.2. Also displayed in the lower right corner is the non-linear cosine curve fit for CBT in subject 06, $R^2 = 0.24$. The circadian phase position in this subject was 6:00 am.

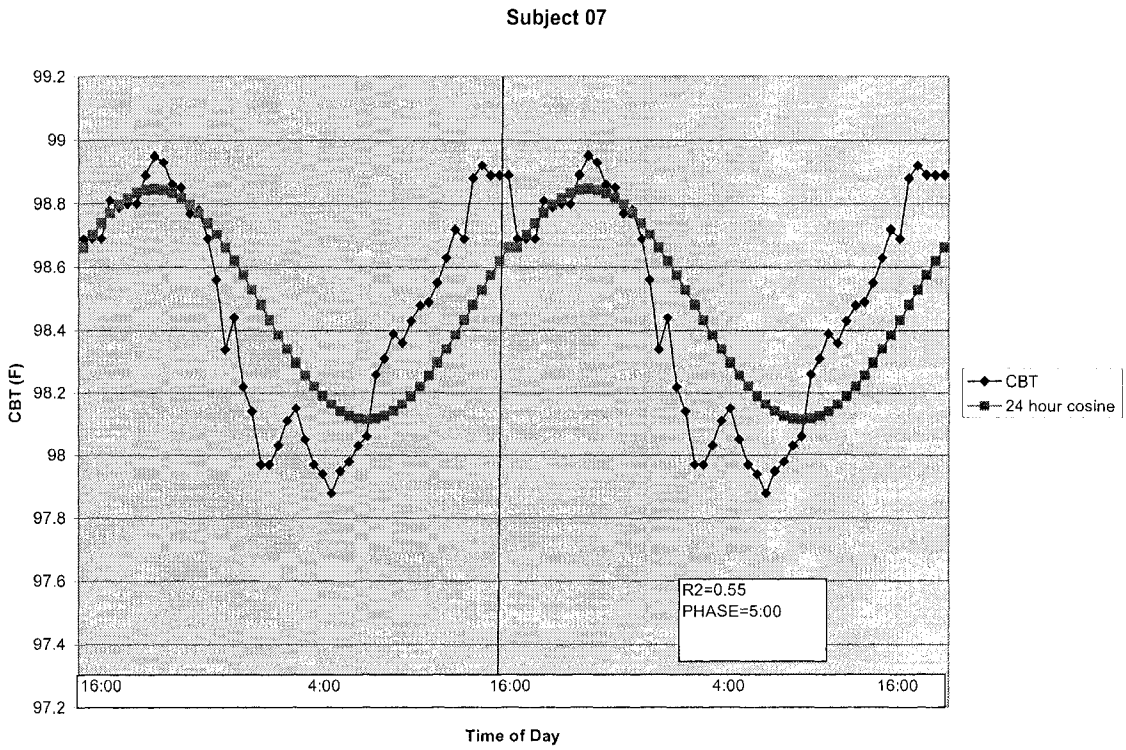

Figure 7. Time course of CBT in subject 07 according to time of day fit to a 24 hour cosine curve. Shown is a double plot of CBT (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBT values (degrees F). The vertical line indicates where the data was folded. Mean CBT=98.5, SD=0.4. Also displayed in the lower right corner is the non-linear cosine curve fit for CBT in subject 07, $R^2 = 0.55$. The circadian phase position in this subject was 5:00 am.

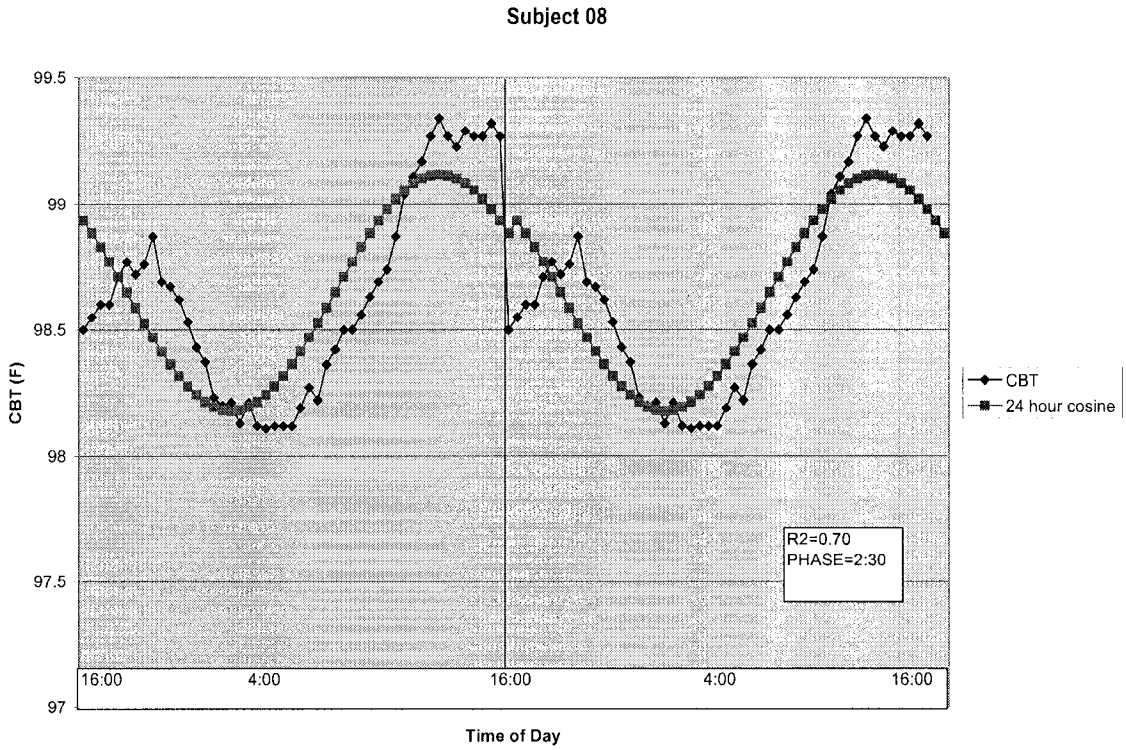

Figure 8. Time course of CBT in subject 08 according to time of day fit to a 24 hour cosine curve. Shown is a double plot of CBT (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBT values (degrees F). The vertical line indicates where the data was folded. Mean CBT=98.6, SD=0.4. Also displayed in the lower right corner is the non-linear cosine curve fit for CBT in subject 08, $R^2 = 0.70$. The circadian phase position in this subject was 2:30 am.

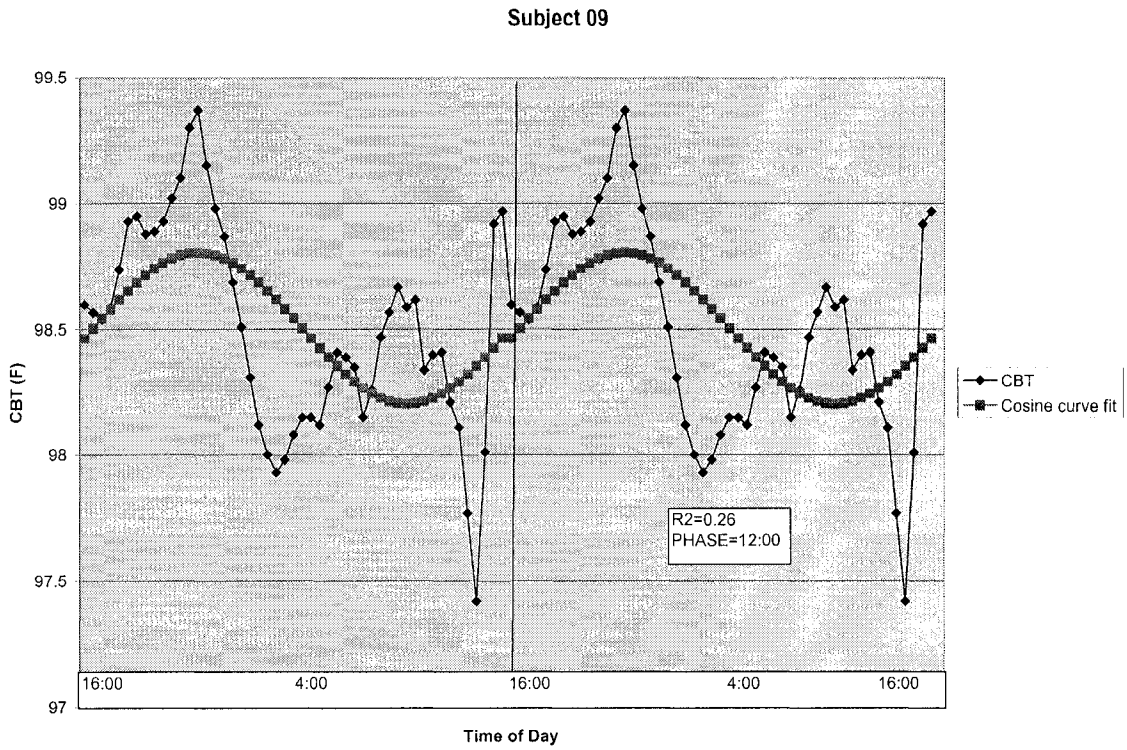

Figure 9. Time course of CBT in subject 09 according to time of day fit to a 24 hour cosine curve. Shown is a double plot of CBT (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBT values (degrees F). The vertical line indicates where the data was folded. Mean CBT=98.5, SD=0.4. Also displayed in the lower right corner is the non-linear cosine curve fit for CBT in subject 09, $R^2 = 0.26$. The circadian phase position in this subject was 12:00 pm.

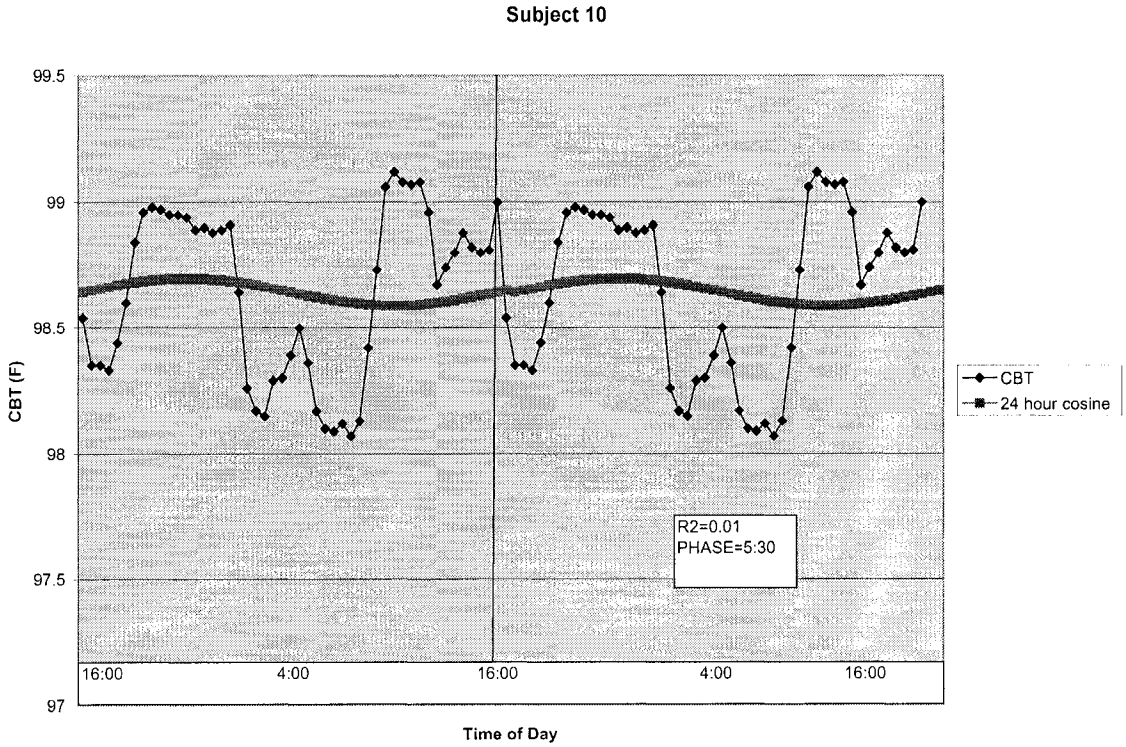

Figure 10. Time course of CBT in subject 10 according to time of day fit to a 24 hour cosine curve. Shown is a double plot of CBT (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBT values (degrees F). The vertical line indicates where the data was folded. Mean CBT=98.6, SD=0.3. Also displayed in the lower right corner is the non-linear cosine curve fit for CBT in subject 10, $R^2 = 0.01$. The circadian phase position in this subject was 5:30 am.

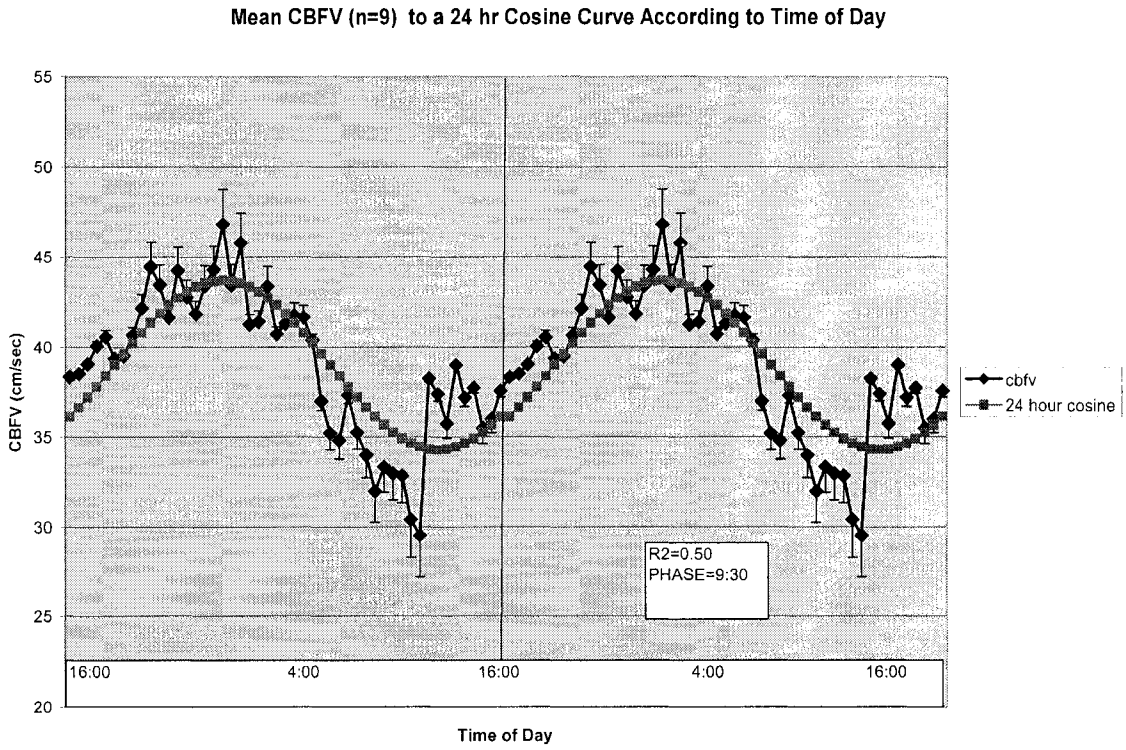

Figure 11. Time course of CBFV across 9 subjects according to time of day fit to a 24 hour cosine curve. Shown is a double plot of the group (n=9) mean levels (+/- standard deviation) of CBFV (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBFV values (cm/sec). The vertical line indicates where the data was folded. Also displayed in the lower right corner is the non-linear cosine curve fit for mean CBFV, $R^2 = 0.50$. The overall mean circadian phase position was 9:30 am.

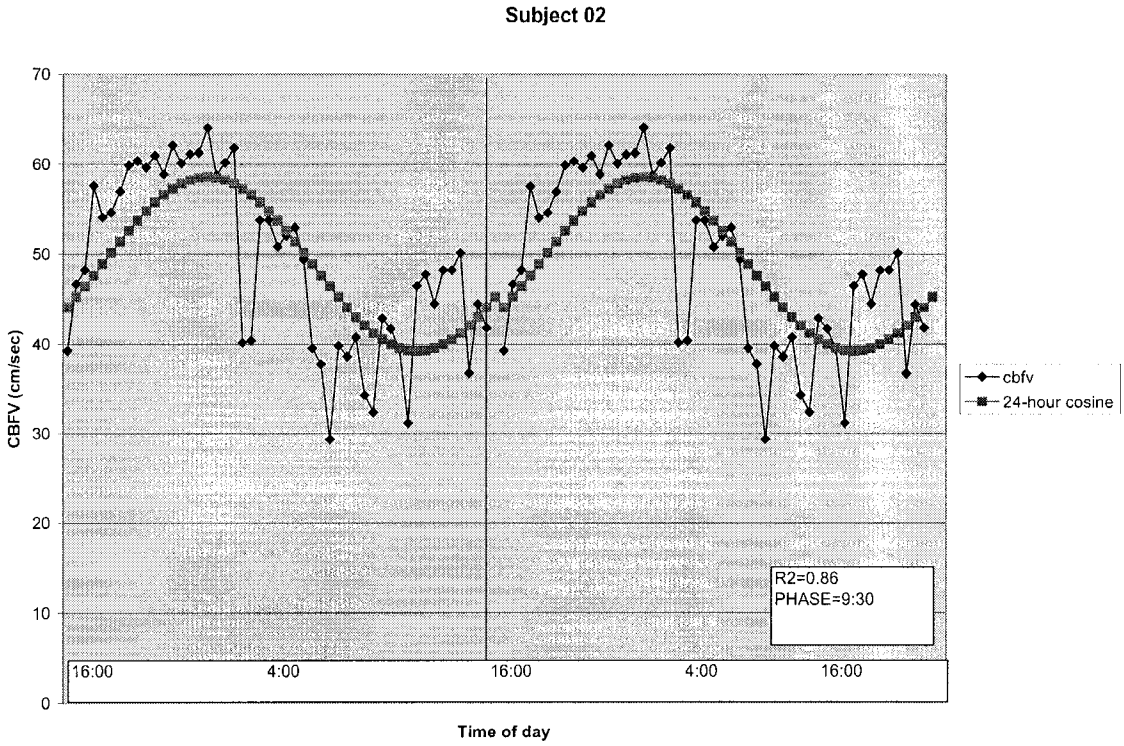

Figure 12. Time course of CBFV in subject 02 according to time of day fit to a 24 hour cosine curve. Shown is a double plot of CBFV (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBFV values (cm/sec). The vertical line indicates where the data was folded. Mean CBFV=48.9, SD=9.61. Also displayed in the lower right corner is the non-linear cosine curve fit for CBFV in subject 02, $R^2 = 0.86$. The circadian phase position in this subject was 9:30 am.

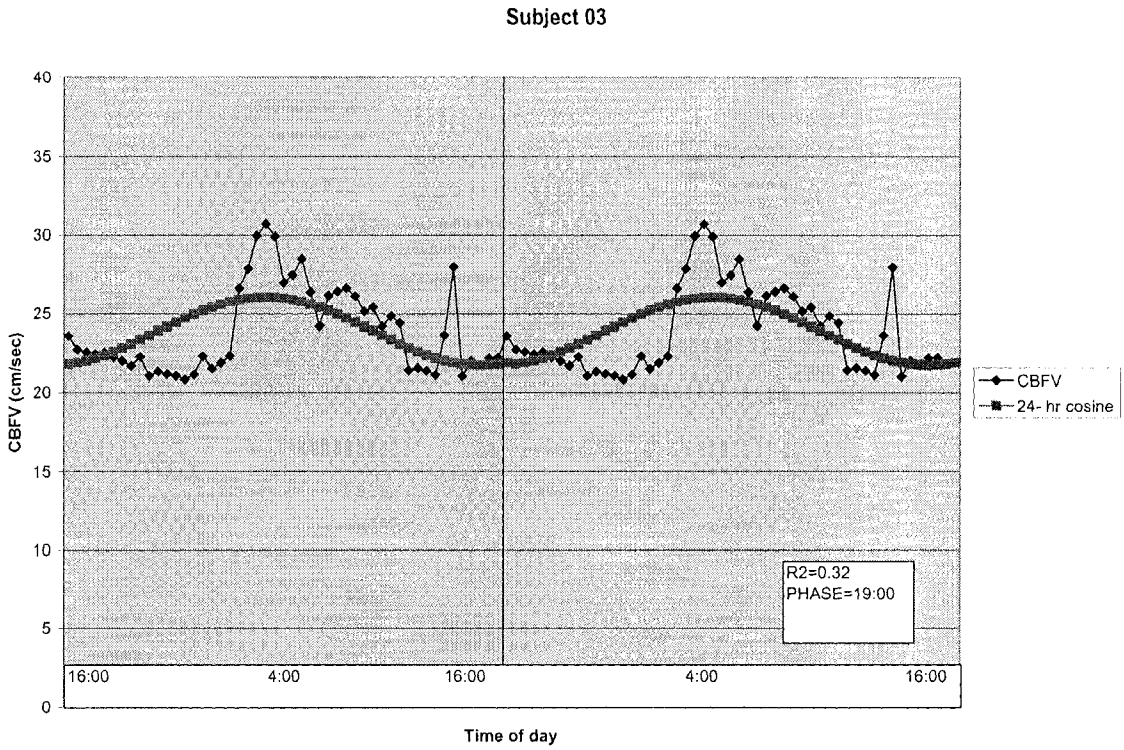

Figure 13. Time course of CBFV in subject 03 according to time of day fit to a 24 hour cosine curve. Shown is a double plot of CBFV (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBFV values (cm/sec). The vertical line indicates where the data was folded. Mean CBFV=23.9, SD=2.8. Also displayed in the lower right corner is the non-linear cosine curve fit for CBFV in subject 03, $R^2 = 0.32$. The circadian phase position in this subject was 19:00.

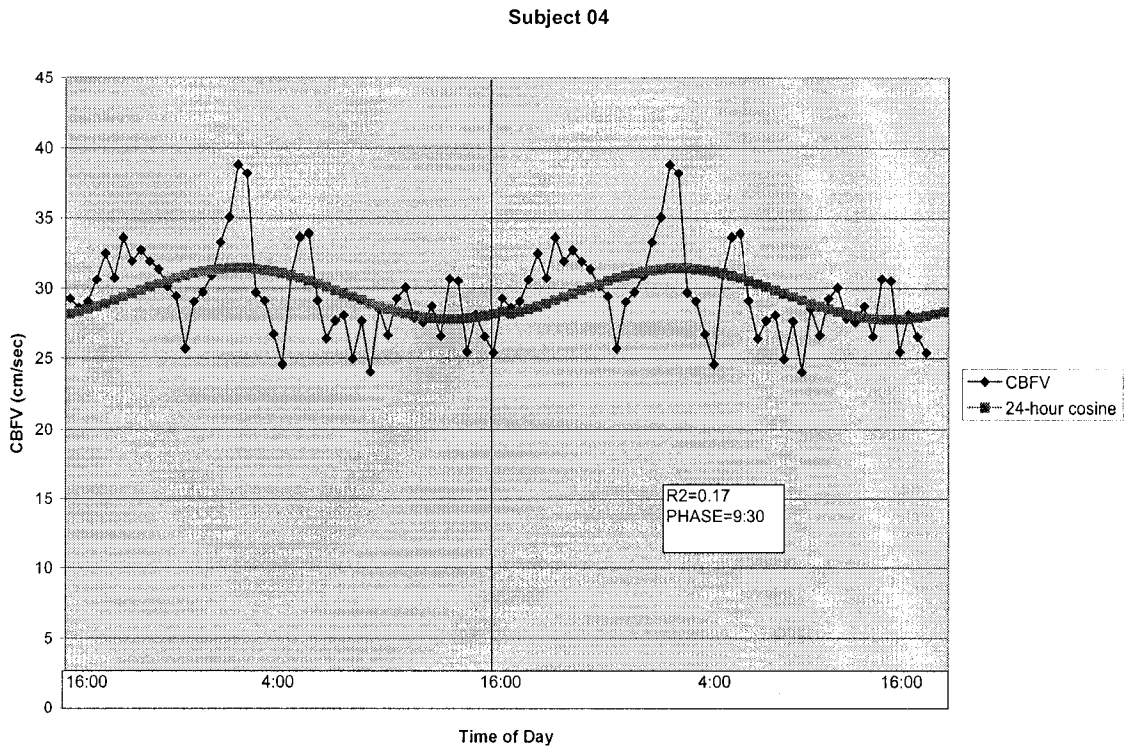

Figure 14. Time course of CBFV in subject 04 according to time of day fit to a 24 hour cosine curve. Shown is a double plot of CBFV (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBFV values (degrees cm/sec). The vertical line indicates where the data was folded. Mean CBFV=29.6, SD=3.2. Also displayed in the lower right corner is the non-linear cosine curve fit for CBFV in subject 04, $R^2 = 0.17$. The circadian phase position in this subject was 9:30.

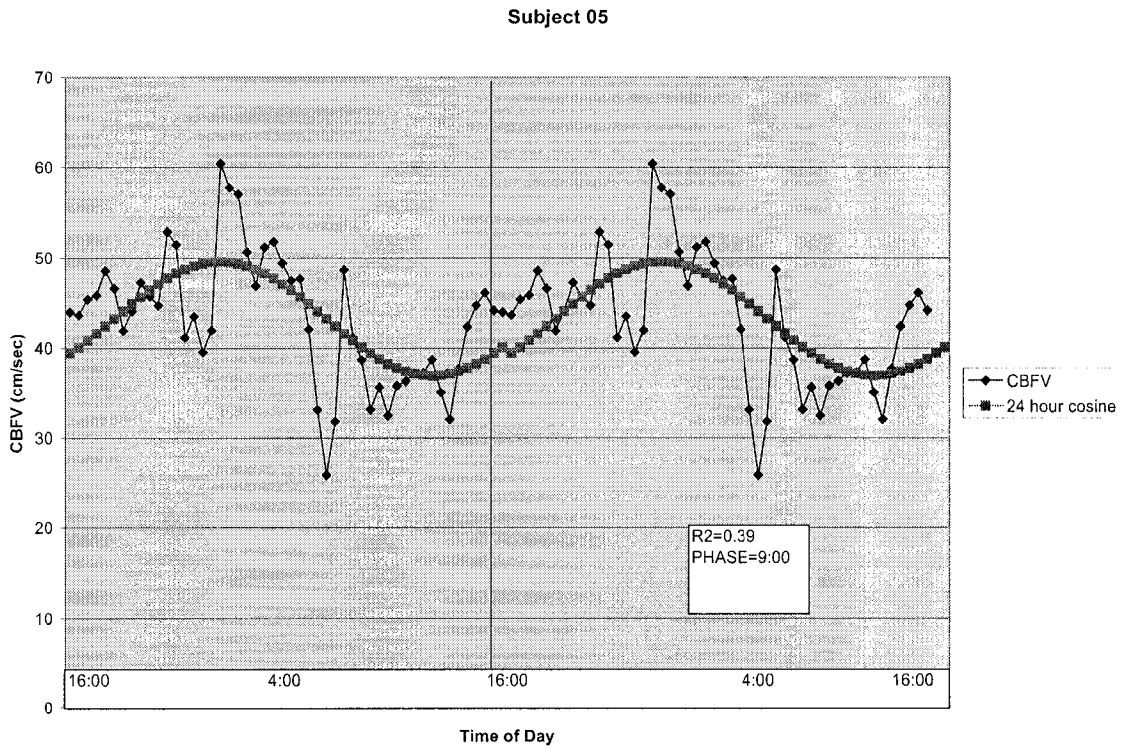

Figure 15. Time course of CBFV in subject 05 according to time of day fit to a 24 hour cosine curve. Shown is a double plot of CBFV (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBFV values (degrees cm/sec). The vertical line indicates where the data was folded. Mean CBFV=43.3, SD=7.3. Also displayed in the lower right corner is the non-linear cosine curve fit for CBFV in subject 05, $R^2 = 0.39$. The circadian phase position in this subject was 9:00.

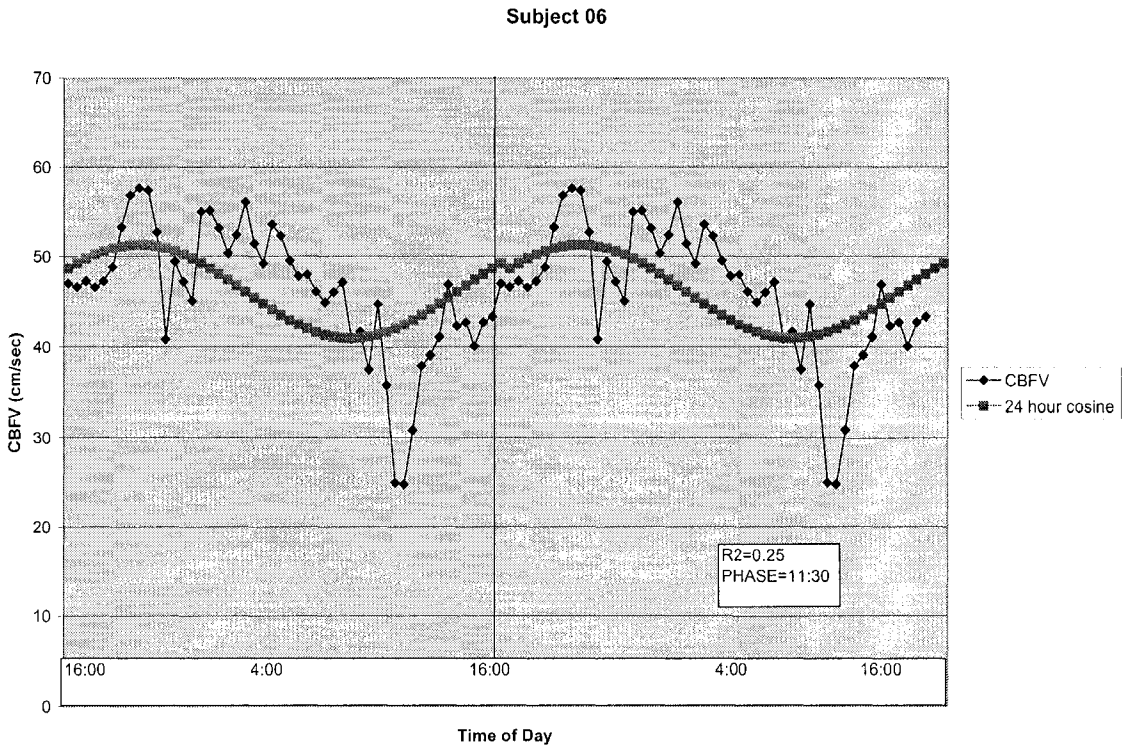

Figure 16. Time course of CBFV in subject 06 according to time of day fit to a 24 hour cosine curve. Shown is a double plot of CBFV (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBFV values (degrees cm/sec). The vertical line indicates where the data was folded. Mean CBFV =46.1, SD=7.4. Also displayed in the lower right corner is the non-linear cosine curve fit for CBFV in subject 06, $R^2 = 0.25$. The circadian phase position in this subject was 11:30.

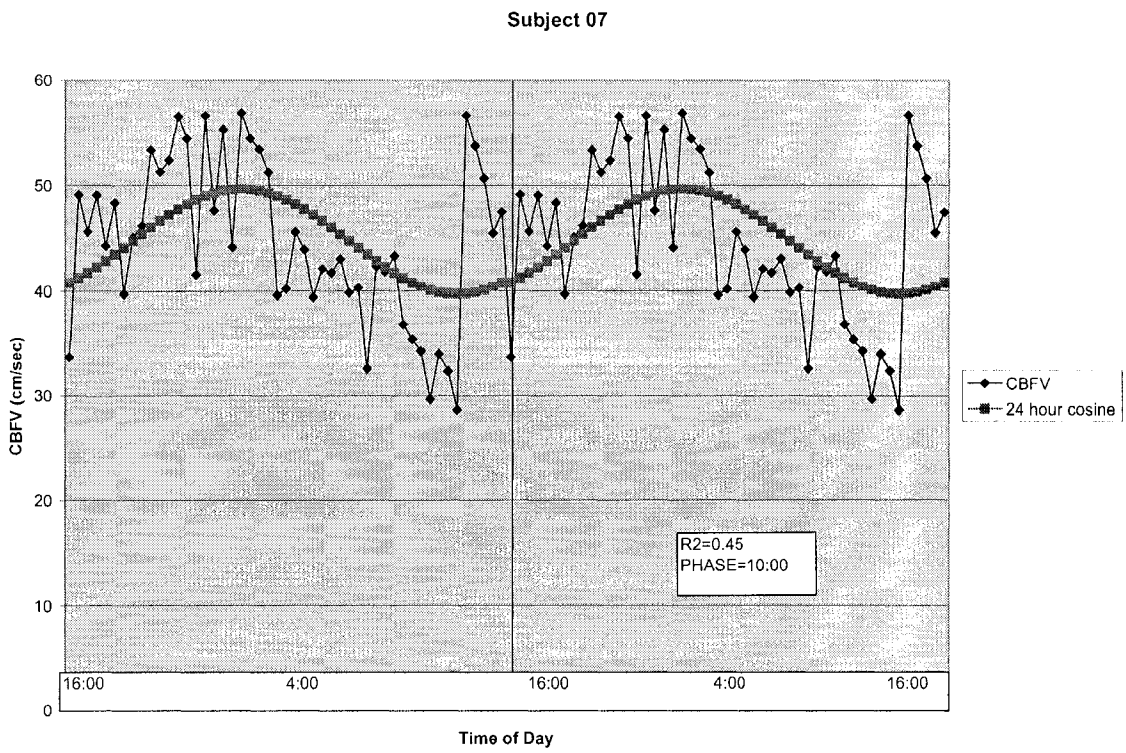

Figure 17. Time course of CBFV in subject 07 according to time of day fit to a 24 hour cosine curve. Shown is a double plot of CBFV (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBFV values (degrees cm/sec). The vertical line indicates where the data was folded. Mean CBFV=44.7, SD=7.7. Also displayed in the lower right corner is the non-linear cosine curve fit for CBFV in subject 07, $R^2 = 0.21$. The circadian phase position in this subject was 11:30.

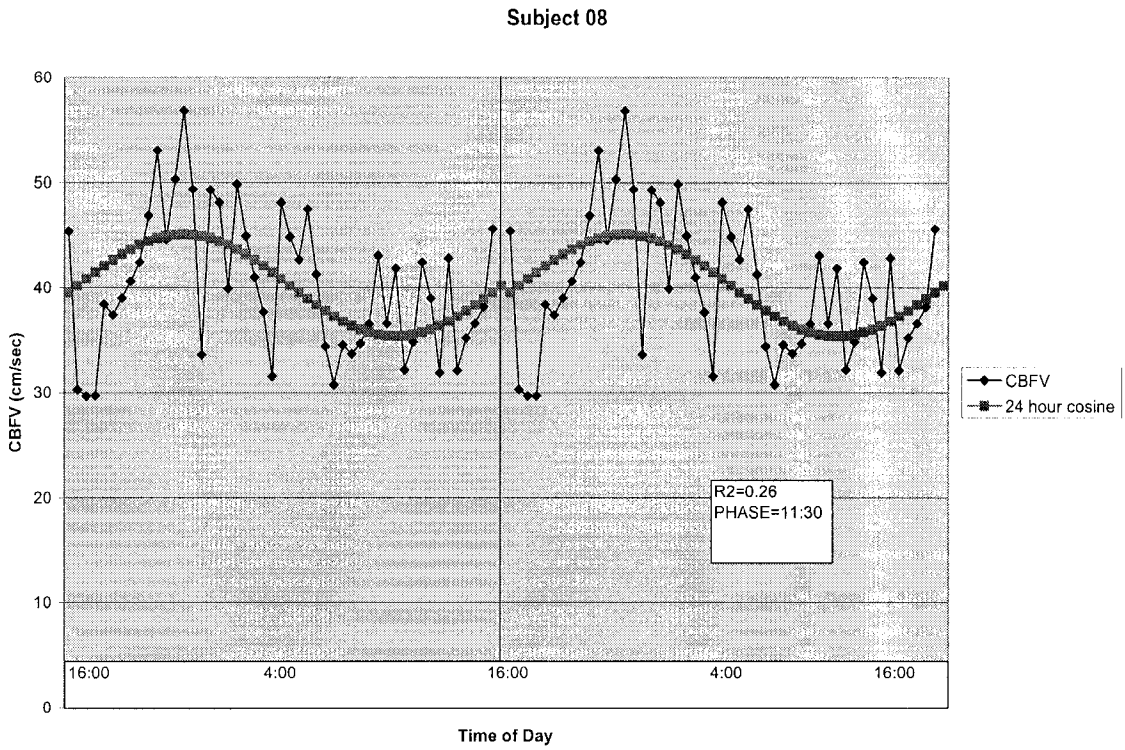

Figure 18. Time course of CBFV in subject 08 according to time of day fit to a 24 hour cosine curve. Shown is a double plot of CBFV (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBFV values (degrees cm/sec). The vertical line indicates where the data was folded. Mean CBFV=40.2, SD=6.72. Also displayed in the lower right corner is the non-linear cosine curve fit for CBFV in subject 08, $R^2 = 0.26$. The circadian phase position in this subject was 11:30.

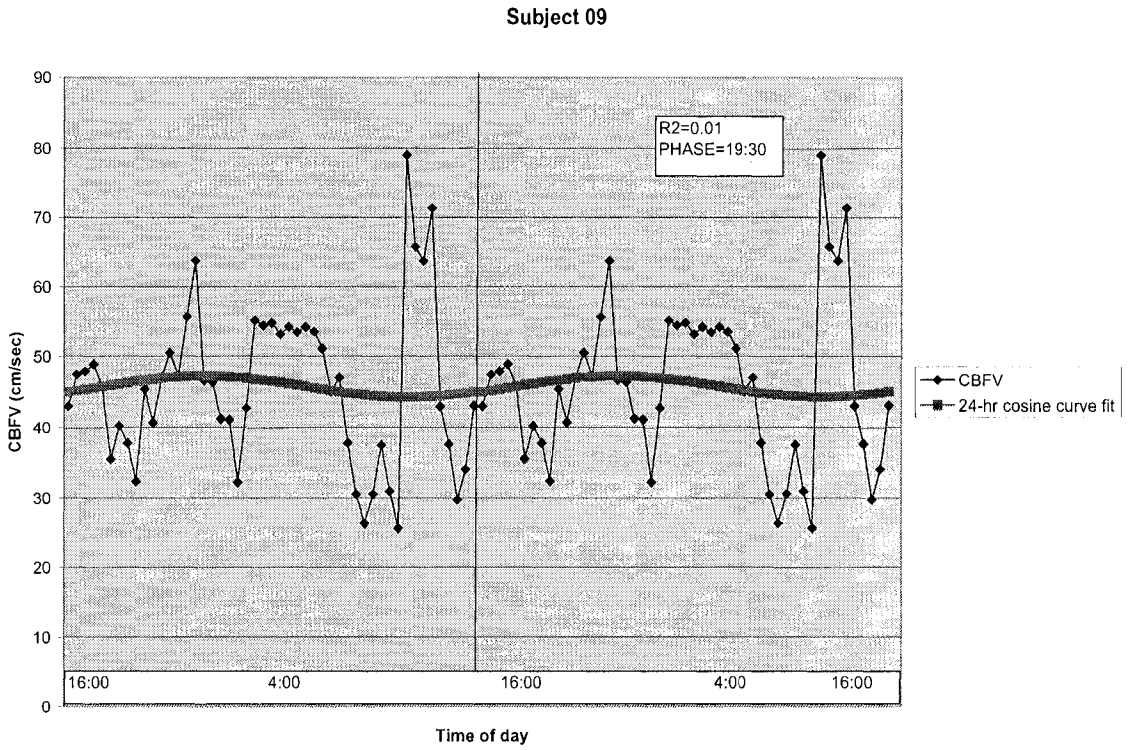

Figure 19. Time course of CBFV in subject 09 according to time of day fit to a 24 hour cosine curve. Shown is a double plot of CBFV (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBFV values (degrees cm/sec). The vertical line indicates where the data was folded. Mean CBFV=45.8, SD=11.4. Also displayed in the upper right corner is the non-linear cosine curve fit for CBFV in subject 09, $R^2 = 0.01$. The circadian phase position in this subject was 19:30.

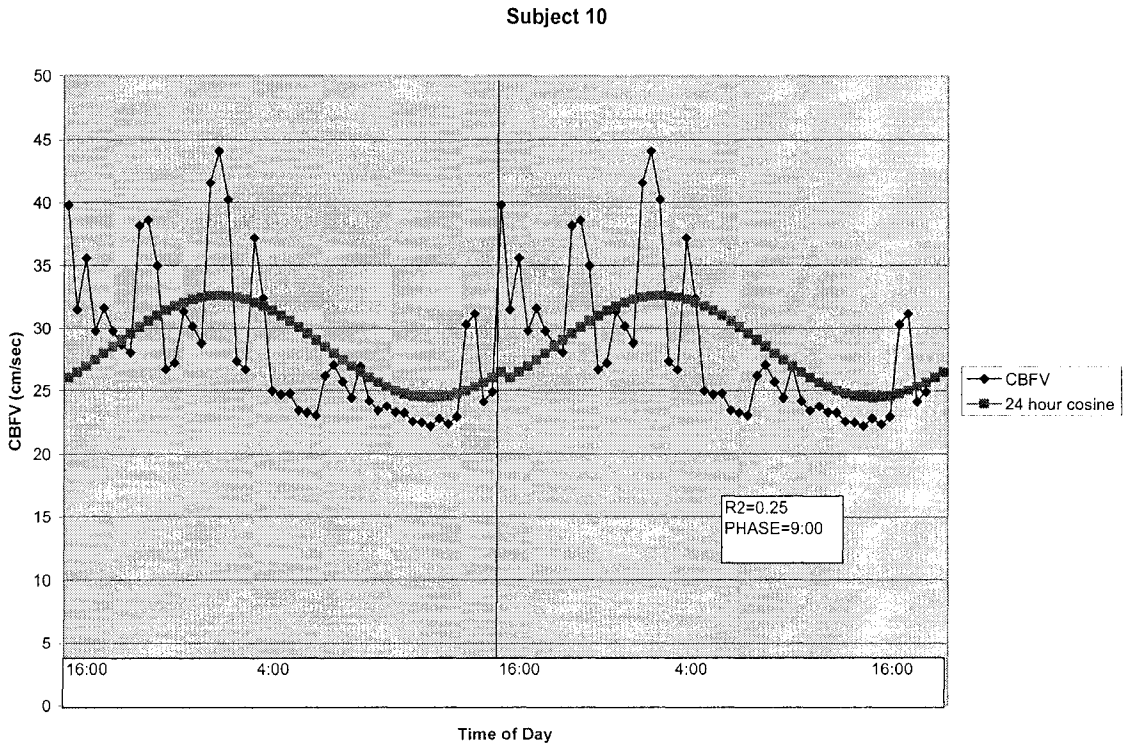

Figure 20. Time course of CBFV in subject 10 according to time of day fit to a 24 hour cosine curve. Shown is a double plot of CBFV (diamonds) fit with a 24-hour cosine curve (squares). Time of day is shown on the abscissa. The ordinate shows CBFV values (degrees cm/sec). The vertical line indicates where the data was folded. Mean CBFV=28.5, SD=5.8. Also displayed in the lower right corner is the non-linear cosine curve fit for CBFV in subject 10, $R^2 = 0.25$. The circadian phase position in this subject was 9:00.

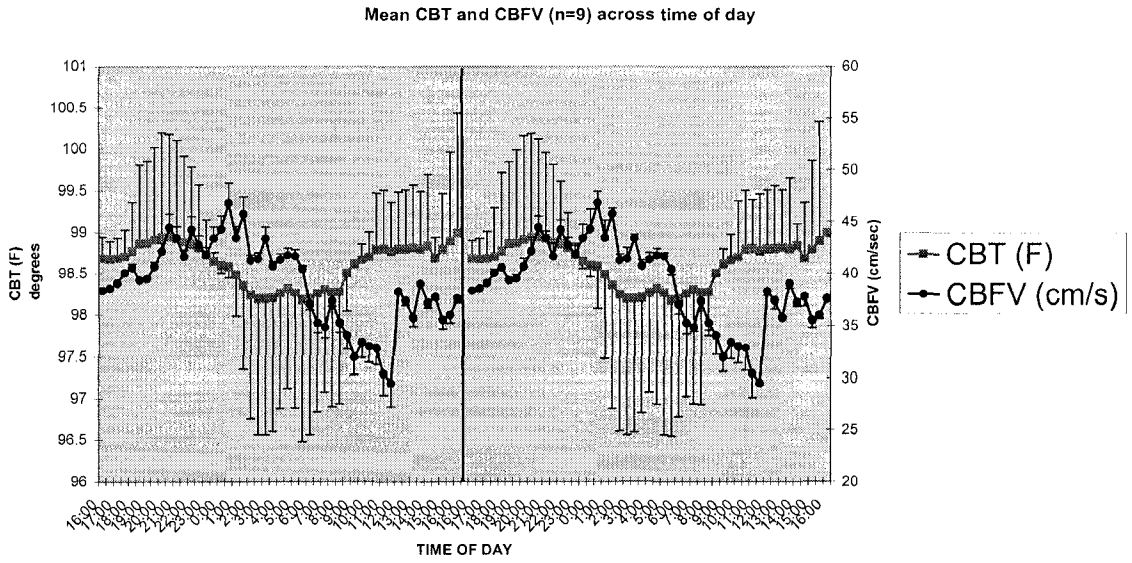

Figure 21. Time course of CBFV and CBT across 9 subjects aligned to time of day. Shown is a double plot of the group (n=9) mean levels (+/- standard deviation) of CBT (squares) and CBFV (circles). Time of day is shown on the abscissa. The ordinate shows CBT values (degrees F) on the left and CBFV (cm/s) on the right. The vertical line indicates where the data was folded.

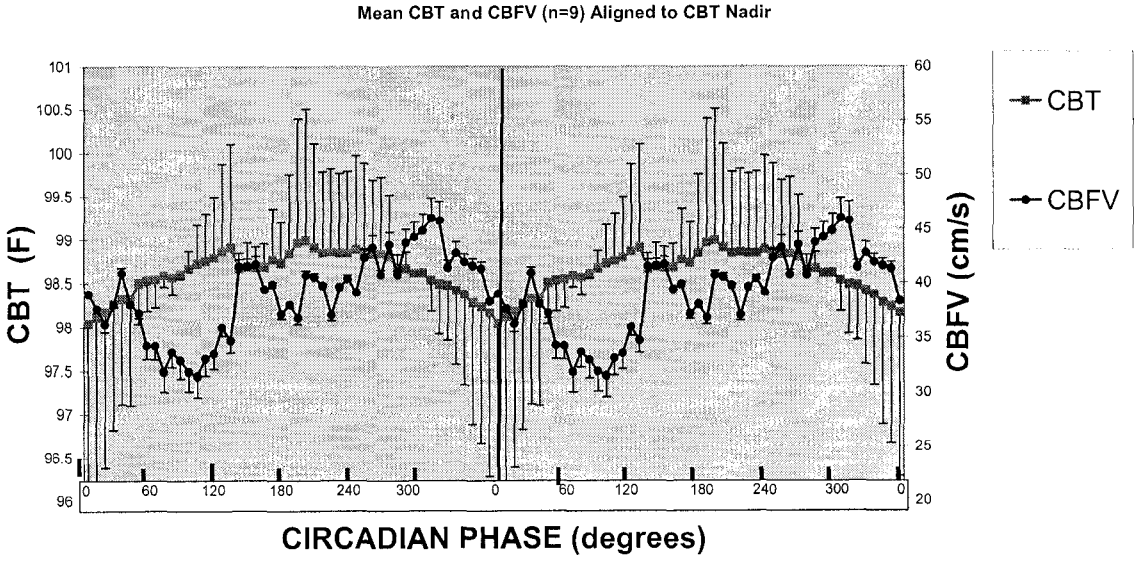

Figure 22. Time course of mean CBFV and mean CBT aligned to the nadir of CBT and then averaged. Shown is a double plot of the group (n=9) mean levels (+/-standard deviation) of CBT (squares) and CBFV (circles) aligned to the phase of the circadian temperature cycle. Circadian time in degrees is shown on the abscissa. The ordinate shows CBT values (degrees F) on the left and CBFV (cm/sc) on the right. The vertical line indicates the CBT nadir.

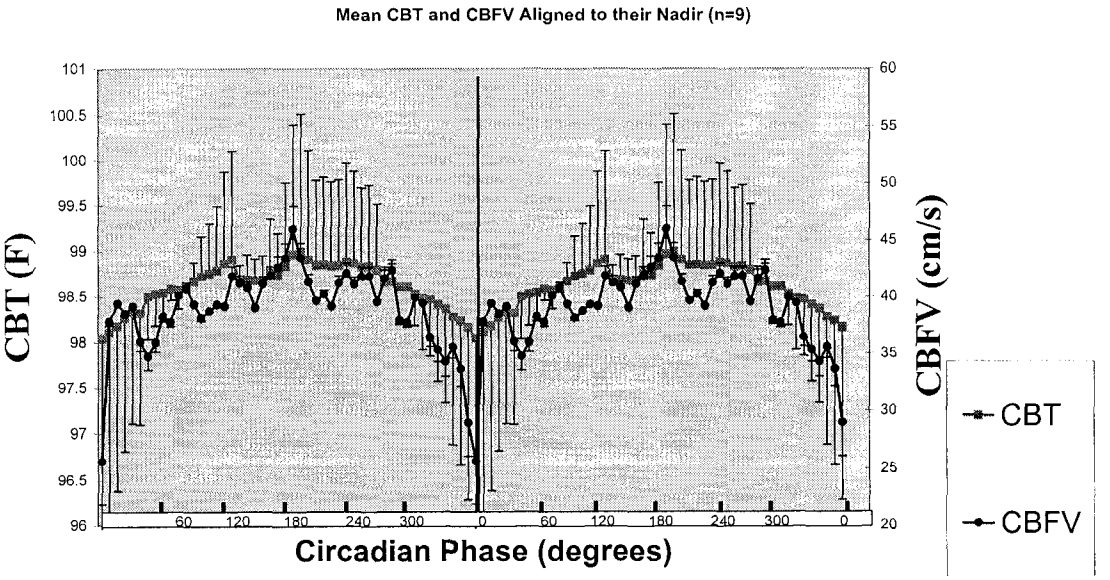

Figure 23. Time course of CBFV nadir shifted to align to CBT nadir
Shown is a double plot of the group (n=9) mean levels (+/-standard deviation) of CBT (squares) and CBFV (circles) when CBFV nadir is aligned to the nadir of the endogenous circadian temperature cycle. Circadian time in degrees is shown on the abscissa. The ordinate shows CBT values (F) on the left and CBFV (cm/s) on the right. The vertical line indicates the nadir of both CBT and CBFV.

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Autobiographical statement

This accomplishment is a childhood dream realized.

My passion for the study of sleep developed at a young age. I remember going to The Cotuit Public Library located in my hometown on Cape Cod, Massachusetts when I was around 10 or 11 years old. I randomly noticed Freud's "Interpretation of Dreams" on a bookshelf. I took the book off of the shelf not knowing who this "Freud" guy actually was and set it down next to my purple Trapper Keeper. I tried to read a few excerpts from the book, but I didn't understand a word of it. So, the book was back in its' rightful place on the bookshelf in a matter of minutes. Throughout my teenage years, I was always interested in books and movies about dreams and nightmares. I was also very intrigued by my own occasional experiences of sleepwalking to the bathroom in the middle of the night and waking up in the shower. During my freshman year in high school, I began flirting with the idea of studying sleep in college. At that time, I didn't know if classes on the subject were even offered. Towards the end of my high school junior year psychology class, my teacher asked us to suggest a final topic to study before the semester ended. The class stayed silent, so I suggested sleep. Unfortunately, our textbooks covered the subject of sleep in only two paragraphs at the end of the book. I left the class with the desire to know more.

When it became time to search for the "perfect college," the usual factors such as reputation, location, and size of the student body were certainly important to me. One of the schools I visited was Simmons College, a small women's college in Boston. My first impression of the school was that it had a good reputation and a great location. The small size of the school also suited me. While taking a tour of the school, the guide showed me a small one-bedroom sleep laboratory. I looked through a one-way mirror and saw a small bed up against the wall in the dark bedroom. There were long colorful wires hanging on the wall and a huge polygraph machine with several tiny dials. I pictured myself working in that sleep lab and using all of the equipment to study sleep. At that point, I had made up my mind that I would be enrolling at Simmons in the fall. While at Simmons, I pursued my interest in sleep under the direction of Dr. Donald Thomas, one of my psychology professors. Dr. Thomas and I shared a mutual interest in sleep and he fostered my desire to pursue a career in the sleep field.

After graduating from Simmons, I took a position at The Harvard Medical School's Laboratory for Circadian Rhythm and Sleep Disorders Medicine. I didn't realize it at the time, but this job served as a gateway into the world of sleep for me. The whirlwind of sophisticated research on sleep and circadian rhythms captivated me. Performing research there fueled my interest and assured me that I had found my career. I heeded the advice of the many other researchers with whom I had the honor to work and followed my dream to The City College of New York to study under Dr. Arthur Spielman. Dr. Spielman has proven to be a brilliant professor, a trusted advisor, and a good friend.

My educational and professional experience, while attending City University of New York, has been the most challenging and difficult task of my life. It has been grueling and it has been humbling. This task has required extreme tenacity and it has left me exhausted. I hope to take full advantage of this opportunity and to do great things in the field of sleep medicine.