AMBULATORY BLOOD PRESSURE MONITORING

Modeling of Circadian Blood Pressure Variation by Square Wave Fitting

Promotiecommissie

Promotor:	Prof. Dr. E.S. Gelsema
Promotor:	Prof. Dr. A.J. Man in 't Veld
Overige leden:	Prof. Dr. Ir. J.H. van Bemmel
	Dr. A.H. van den Meiracker
	Prof. Dr. M.A.D.H. Schalekamp

The studies presented in this thesis were carried out in the Department of Internal Medicine I of the University Hospital 'Dijkzigt' and in the Department of Medical Informatics, Erasmus University Rotterdam

The investigations were financially supported by the Netherlands Heart Foundation (NHS 86.070) and by the Cardiovascular Reseach Foundation Rotterdam. Financial support by the Netherlands Heart Foundation for the publication of this thesis is gratefully acknowledged.

Voor mijn ouders

Aan Kristine

Table of Contents

Chapter 1

Introduction

Introduction

Clinical Importance of Diurnal Blood Pressure Patterns

The conventional cuff measurement of blood pressure as measured in the doctor's office is the cornerstone of our knowledge of the risks associated with hypertension[1]. In the period from 1940 to 1970, there was an increasing awareness of the intrinsic variability of blood pressure[2,3] and the fact that the conventional clinic measurements of pressure may in some patients be quite unrepresentative of their overall levels of pressure[3-5]. This lack of precision and accuracy of the clinic blood pressure has since then been confirmed by many investigators using ambulatory blood pressure monitoring[6-8]. These studies resulted amongst others in the concept of "white coat hypertension"[9] , which is by now a well-recognized diagnostic artifact and not an independent risk factor for cardiovascular disease (see Verdecchia[8] and the references therein). The 24-hour average ambulatory pressure appears to be a better reproducible parameter than clinic blood pressure[10-13], and it correlates better with the presence and severity of cardiovascular morbidity[8,14-17] and with cardiovascular mortality[8]. For these reasons, ambulatory blood pressure monitoring is increasingly used, not only in research but also in clinical practice[18].

The technique of ambulatory blood pressure monitoring yields large numbers of measurements (typically 50 to 100 for cuff and over 100,000 for continuous measurements), which include blood pressure values during the full range of daily activities. This vast amount of data has raised the question which measure of pressure may give the best prediction of risk. There may not be a simple answer to this question because it is conceivable that different aspects of blood pressure might be responsible for different pathogenic effects, or they may be symptomatic for different defects in the blood pressure regulation[19]. It may be, for example, that the average level is more important in producing left ventricular hypertrophy, whereas the peaks in blood pressure trigger the rupture of an atheromatous plaque.

The most prominent and consistent finding in the 24-hour blood pressure pattern is the nocturnal decrease in blood pressure. The mechanism behind this so-called *diurnal* or *circadian blood pressure variation* has been extensively discussed[19]. Although a sinusoidal, intrinsic rhythm of circadian blood pressure regulation has been postulated in man[20], experiments in shift workers[21-23] , in subjects immobilized by orthopedic plaster casts[24], in subjects confined to bed[25,26], and in subjects in whom the precise pattern of daily activities was known[27] did not confirm the presence of such a rhythm and these

observations have convincingly demonstrated that the daily pattern of activity and sleep is by far the most prominent denominator of the diurnal blood pressure variation.
Cross-sectional studies have demonstrated that attenuation of the diurnal blood pressure variation is related to target organ damage. There is evidence of increased left ventricular mass[28-32], more advanced silent cerebrovascular damage[33], and a more frequent history of stroke[34] in hypertensive subjects in whom the nocturnal decrease in blood pressure is attenuated in comparsion with subjects having a normal circadian blood pressure variation. It has been argued that the worse outcome in subjects in whom the nocturnal decrease in blood pressure is attenuated may be more closely associated with a higher average blood pressure over the 24 hours than with an altered diurnal blood pressure rhythm itself [35]. In a recent longitudinal study [8], however, the cardiovascular morbidity and mortality remained increased in subjects having an attenuated diurnal variation of blood pressure in comparison with subjects having a normal diurnal variation, even after adjustment for the average 24 hour blood pressure values.

Definitions of Diurnal Blood Pressure Variation

Although the association of attenuation of the diurnal blood pressure variation with cardiovascular morbidity and mortality is now well established, the nocturnal blood pressure fall itself appears to be ill-defined. Van Ittersum[36] recently reviewed 42 studies using 24-hour ambulatory blood pressure monitoring. In 13 studies, the nocturnal blood pressure fall was calculated as the difference of blood pressure during sleep and awakeness, whereas the 29 other studies employed the difference of average blood pressure between fixed clock time periods. In the latter studies, 17 different time periods had been used to define the day and the night period. Fagard et al[37] found nine different definitions for these periods in a meta-analysis of the relationships of daytime and night-time pressures and left ventricular hypertrophy. Others counted 12 different time schedules[38]. Surprisingly, the definition of the night and the day period may even differ between major studies of the same group of investigators[8,30]. The various definitions of the day and night period probably reflect the wide spectrum of investigated patient groups and the expectation of the investigators about the sleep and activity patterns in the particular group of subjects under study. The different definitions persist in spite of an attempt of standardization in 1990 by the International Consensus Conference on Indirect Ambulatory Blood Pressure Monitoring[39].
Although many different definitions of the daytime and nighttime are used, this does not mean that a proper definition of these periods is irrelevant. Various investigators have demonstrated that the day-night blood pressure difference depends on the definition chosen[36,38,40], and this observation precludes comparison of results from studies employing different calculation schemes. The use of a fixed-time method within a group of subjects is also not without problems, since there may be considerable differences in activity patterns between subjects. Different investigators[36,38,41,42] agree that important differences from the actual awake or asleep blood pressures, or both, may occur when daytime and nighttime blood pressures are assessed by fixed-time methods, particularly when sleeping habits are discordant from arbitrarily set limits or when the morning and evening transition periods are not excluded. These findings probably also explain that the nocturnal blood

pressure fall within subjects assessed by a fixed clock time calculation scheme is poorly reproducible[40,43-46]. A method that adapts to the different sleep and activity patterns may therefore be useful for an analysis of the diurnal blood pressure variation that is more consistent than fixed clock-time calculation schemes.
A consensus concerning how the day-night blood pressure should be calculated is desirable because of the possible differences in the strength of the relationships of the various pressures with target organ damage and cardiovascular risk. The variety of definitions hampers the comparability of studies and might preclude possible meta-analysis of the available material from several sources. It has recently been suggested that the most meaningful method for defining the day and the night period is by using the subjects' reports of waking and sleeping times[47]. The use of diaries, however, is cumbersome. Of more fundamental importance is that this method cannot be used for possible re-analysis of studies in which these times have not been noted and in which other definitions of the day and night have been used.

Aim of the Present Thesis

The present thesis introduces a method that estimates the nocturnal blood pressure change from the blood pressure profile itself, without using clock time information. The method is based on a model of 24-hour blood pressure consisting of two complementary periods of constant pressure, a so-called square wave. Determination of the transience times between the periods is performed individually, using a least square error criterion. The method is evaluated for continuous intra-arterial blood pressure registration and compared to other methods which model the circadian variation. The applicability of the method under various conditions is investigated in artificial blood pressure profiles by means of a Monte Carlo experiment, and subsequently tested in profiles recorded by cuff measurement in subjects having a normal or an attenuated diurnal blood pressure variation. The method is applied to intra-arterial blood pressure registrations recorded in cardiac transplant recipients, and the attenuated nocturnal blood pressure fall in these subjects is hemodynamically characterized.
The following questions were investigated.

- What is the performance of the blood pressure recording system and analysis software?
- How does the square wave model compare to other models of circadian blood pressure variation, and which model best describes the circadian rhythm?
- What is the dependency of the square wave parameter estimation on the measurement frequency, the between-measurement variation and the magnitude of the diurnal blood pressure variation?
- Can the square wave fit be applied to 24-hour blood pressure registrations obtained by means of non-invasive measurement, and can it be used in outpatients in whom daily activities are less restricted than in hospitalized subjects?
- Does the square wave fit estimation of the period of low pressure correspond with the period of bed rest?
- What is the hemodynamic basis of the attenuated circadian variation of blood pressure in cardiac transplant recipients?

References

1. Dawber TR: The Framingham Study. The Epidemiology of Atherosclerotic Disease. Cambridge: Harvard University Press, 1980
2. Armitage P, Rose GA: The variability of measurements of casual blood pressure. I. A laboratory study. Clin Sci 1966;30:325-335
3. Bevan AT, Honour AJ, Stott FW: Direct arterial pressure recording in unrestricted man. Clin Sci 1969;39:329-344
4. Ayman D, Goldshine AD: Blood pressure determinations by patients with essential hypertension: difference between clinic and home readings before treatment. Am J Med Sci 1940;200:465-474
5. Kain HK, Hinman AT, Sokolow M: Arterial blood pressure measurements with a portable recorder in hypertensive patiens. I. Variability and correlation with "casual" pressure
6. Van Montfrans GA: Continuous ambulatory blood pressure registration in uncomplicated hypertension. Cuff-responders and blood pressure variability. Amsterdam: University of Amsterdam, 1984. Dissertation.
7. Manica G, Grassi G, Pomidossi G, Gregorini L, Berinieri G, Parati G, Ferrari A, Zanchetti A: Effects of blood-pressure measurement by the doctor on patient's blood pressure and heart rate. Lancet 1983;ii:695-698
8. Verdecchia P, Porcellati C, Schillaci G, Borgioni C, Ciucci A, Battistelli M, Guerrieri M, Gatteschi C, Zampi I, Santucci A, Santucci C, Reboldi G: Ambulatory blood pressure: an independent predictor of prognosis in essential hypertension. Hypertension 1994;24:793-801
9. Kleinert HD, Harshfield GA, Pickering TG: What is the value of home blood pressure measurement in patients with mild hypertension? Hypertension 1984;6: 574-578
10. James GD, Pickering TG, Yee LS, Harshfield GA, Riva S, Laragh JH: The reproducibility of average ambulatory, home, and clinic pressures. Hypertension 1988;11:545-549
11. Coats AJS, Conway J, Somers VK, Isea JE, Sleight P: Ambulatory blood pressure monitoring in the assessment of antihypertensive therapy. Cardiovasc Drug Ther 1989;3:303-311
12. Trazzi S, Mutti E, Frattola A, Imholz B, Parati G, Mancia G: Reproducibility of non-invasive and intra-arterial blood pressure monitoring: implications for studies on antihypertensive treatment. J Hypertens 1991;9:115-119
13. Coats AJS, Radaelli A, Clark SJ, Conway J, Sleight P: The influence of ambulatory blood pressure monitoring on the design and interpretation of trials in hypertension. J Hypertens 1992;10:385-391
14. Devereux RB, Pickering TG, Harshfield GA, Kleinert HD, Denby L, Clark L, Pregibon D, Jason M, Kleinert B, Borer JS, Laragh JH: Left ventricular hypertrophy in patients with hypertension: Importance of blood pressure response to regularly recurring stress. Circulation 1983;68:470-476

15. Drayer JIM, Weber MA, DeYoung JL: Blood pressure as a determinant of cardiac left ventricular muscle mass. Arch Intern Med 1983;143:90-92
16. Verdecchia P, Schillaci G, Boldrini F, Guerrieri M, Gatteschi C, Benemio G, Porcellati C: Risk stratification of left ventricular hypertrophy in systemic hypertension using noninvasive ambulatory blood pressure monitoring. Am J Cardiol 1990;66:583-590
17. Devereux RB, Pickering TG: Relationship between the level, pattern and variability of ambulatory blood pressure and target organ damage in hypertension. J Hypertens 1991;9(suppl 8):S34-S38
18. Fagard R, Brguljan J, Thijs L, Staessen J: Prediction of the actual awake and asleep blood pressures by various methods of 24 h pressure analysis. J Hypertens 1996; 14:557-563
19. Pickering TG: The clinical significance of diurnal blood pressure variation. Dippers and nondippers. Circulation 1990;81:700-701. Editorial comment
20. Halberg F, Halberg E, Halberg J, Halberg F: Chronobiologic assessment of human blood pressure variation in health and disease. In: Weber MA, Drayer JIM, eds. Ambulatory blood pressure monitoring. Darmstadt: Steinkopff, 1984:137-156
21. Sundberg S, Kohvakka A, Gordin A: Rapid reversal of circadian blood pressure rhythm in shift workers. J Hypertens 1988;6:393-396
22. Baumgart P, Walger P, Fuchs G, Dorst KG, Vetter H, Rahm KH: Twenty-four hour blood pressure is not dependent on endogenous circadian rhythm. J Hypertens 1989;7:331-396
23. Chau NP, Mallion JM, de Gaudemaris R, Ruche E, Siche JP, Pelen, O, Mathern G: Twenty-four-hour blood pressure in shift workers. Circulation 1989;80:341-347
24. Athanassiadis D, Draper GJ, Honour AJ, Cranston WI: Variability of automatic blood pressure measurement over 24 hour periods. Clin Sci 1969;36:147-156
25. Mann S, Millar-Craig MW, Melville DI, Balasubramanian V, Raftery EB: Physical activity and the circadian rhythm of blood pressure. Clin Sci 1979;57:291S-294S
26. Van den Meiracker AH, Man in 't Veld AJ, Ritsema van Eck HJ, Wenting GJ, Schalekamp MADH: Determinants of short-term blood pressure variability: Effects of bedrest and sensory deprivation in essential hypertension. Am J Hypertens 1988; 1:22-26
27. Clark LA, Denby L, Pregibon D, Harshield GA, Pickering TG, Blanks S, Laragh JH: A quantitative analysis of the effects of activity and time of day on the diurnal variation of blood pressure. J Chron Dis 1987;40:671-681
28. Kobrin I, Oigman W, Kumar A, Ventura HO, Messerli Fh, Frohlich ED, Dunn FG. Diurnal variation of blood pressure in elderly patients with essential hypertension. J Am Geriatr Soc 1984;32:896-899
29. Lang RM, Borow KM, Weinert L, Bednarz J, Neumann A, Nelson K, David D, Murphy MB: Circadian blood pressure patterns in hypertensive patients: importance of nocturnal blood pressure. Circulation 1988;78(suppl II):517. Abstract
30. Verdecchia P, Schillaci G, Guerrieri M, Gatteschi C, Benmio G, Boldrini F, Porcellati C: Circadian blood pressure changes and left ventricular hypertrophy in hypertension. Circulation 1990;81:528-536

31. Rizzoni D, Muiesan ML, Montani G, Zulli R, Calebich S, Agabiti-Rosei E. Relationship between initial cardiovascular structural changes and daytime and nighttime blood pressure monitoring. Am J Hypertens 1992;5:180-186
32. Palatini P, Penzo M, Racioppa A, Zugno E, Guzzardi G, Anaclerio A, Pessina AC: Clinical relevance of nighttime blood pressure and of daytime blood pressure variability. Arch Int Med 1992;152:1855-1860
33. Shimada K, Kawamoto A, Matsubayashi K, Nishinaga M, Mimura S, Ozawa T. Diurnal blood pressure variations and silent cerebrovascular damage in elderly patients with hypertension. J Hypertens 1992;10:875-878
34. O'Brien E, Sheridan H, O'Malley K: Dippers and non-dippers. Lancet 1988;2:397. Letter
35. Pickering TG, James GD: Determinants and consequences of the diurnal rhythm of blood pressure. Am J Hypertens 1993;6:166S-169S
36. Van Ittersum FJ, IJzerman RG, Stehouwer CDA, Donker AJM: Analysis of twenty-four-hour-ambulatory blood pressure monitoring: what time period to assess blood pressures during waking and sleeping? J Hypertens 1995;13: 1053-1058
37. Fagard R, Staessen JA, Thijs L: The relationship between left ventricular mass and daytime and night-time blood pressures: a meta-ananlysis of comparative studies. J Hypertens 1995;13:823-829
38. Peixoto-Filho AJ, Mansoor GA, White WB: Effects of actual versus arbitrary awake and sleep times on analyses of 24-h blood pressure. Am J Hypertens 1995;8: 676-680
39. The Scientific Committee: Consensus document on non-invasive ambulatory blood pressure monitoring J Hypertens 1990;8(suppl 6):S135-S140
40. Van der Steen M, den Arend J, Graafsma S, Lenders JWM, Thien Th: Nocturnal blood pressure fall depends on the period chosen. J Hypertens 1996;14:679-680
41. Gatzka CD, Schmieder RE: Improved classification of dippers by individual analysis of ambulatory blood pressure profiles. Am J Hypertens 1995;8:666-671
42. Rosansky SJ, Menackery SJ, Wagner CM, Hackson K: The effect of sleep intervals on analysis of 24-h ambulatory blood pressure data. Am J Hypertens 1995;8:672-675
43. Verdecchia P, Schillaci G, Boldrini F, Guerrieri M, Zampi I, Porcellati C. Quantitative assessment of day-to-night spontaneous variability in non-invasive ambulatory blood pressure measurements in essential hypertension. J Hypertens 1991;9(suppl 6):S322-S323
44. Wong Chung MY, Smits P, Lenders JWM, Thien Th: Reproducibility of the blood pressure fall at night in healthy normotensive volunteers. J Hypertens 1991;9(suppl 6):S324-S325
45. James MA, Fotherby MD, Potter JF: Reproducibility of the circadian systolic blood pressure variation in the elderly. J Hypertens 1995;13:1097-1103
46. Stanton A, Cox J, Atkins N, O'Malley K, O'Brien E: Cumulative sums in quantifying circadian blood pressure patterns. Hypertension 1992;19:93-101
47. Pickering TG: How should the diurnal changes of blood pressure be expressed? Am J Hypertens 1995;8:681-682

Chapter 2

The Blood Pressure Recording System

Introduction

In the present studies, continuous 24-hour ambulatory blood pressure was measured in the brachial artery of the non-dominant arm with an Oxford Medilog blood pressure recording system. The system consists of an intra-arterial cannula connected by a manometer tubing to a transducer perfusion unit and a miniature tape recorder. The transducer and recorder are worn on the chest at heart level and allow free movement of the subject under study. Various groups have used this blood pressure recording system for research purposes[1-3]. Van Montfrans introduced the system in The Netherlands, and provided an elaborate description in his thesis[4]. This chapter describes the blood pressure measuring and recording apparatus, and the recording procedure as it is performed in our department. The chapter finishes with a descripiton of the technical characteristics of the system as it was used by us.

Catheter

The catheter system consists of a Teflon cannula connected to the transducer perfusion unit by means of a manometer tubing. The disposable cannula (Seldicath-cannula, Laboratoire Plastimed, Saint-Leu-la-Forêt, France) has a length of 11 cm with an external diameter of 1.0 mm. After cannulation of the brachial artery, the cannula is connected with Luer connectors to the transducer perfusion unit by means of a 70 cm long manometer tubing with an internal diameter of 0.6 mm. The catheter system is filled with a heparinized isotonic saline solution.

Transducer Perfusion System

The transducer perfusion unit (Brunel Laboratories, Oxford, Oxfordshire, U.K.) is depicted in Figure 1. This model was introduced in 1978[2]. It consists of an epoxy resin cast body in which a transducer and a flushing device are fitted. The miniature semiconductor strain-gauge pressure transducer (AE 831 RV Class 1, Aksjeselskapet Mikro-Elketronikk, Horten, Norway) converts the blood pressure signal to an electric signal that is subsequently recorded. The transducer has a rated pressure range of 0 to 760 mmHg (0 to 1.0 Bar). Non-linearity and hysteresis are smaller than 0.5% of the full scale, and thermal zero shift is smaller than ±0.4 mmHg/°C. The transducer is fitted together with its electronic circuit over a small volume well in the body of the perfusion unit. The power is supplied by the recording system. The frequency characteristic of the catheter transducer manometer system is flat to 15 Hz (±2 dB), and the resonance frequency is approximately 20 Hz.

Continuous flushing of the intra-arterial catheter by a heparinized saline solution prevents that the cannula becomes occluded by blood clots. The reservoir of the transducer perfusion unit has a volume of 40 mL. It is filled with a sterilized heparinized isotone

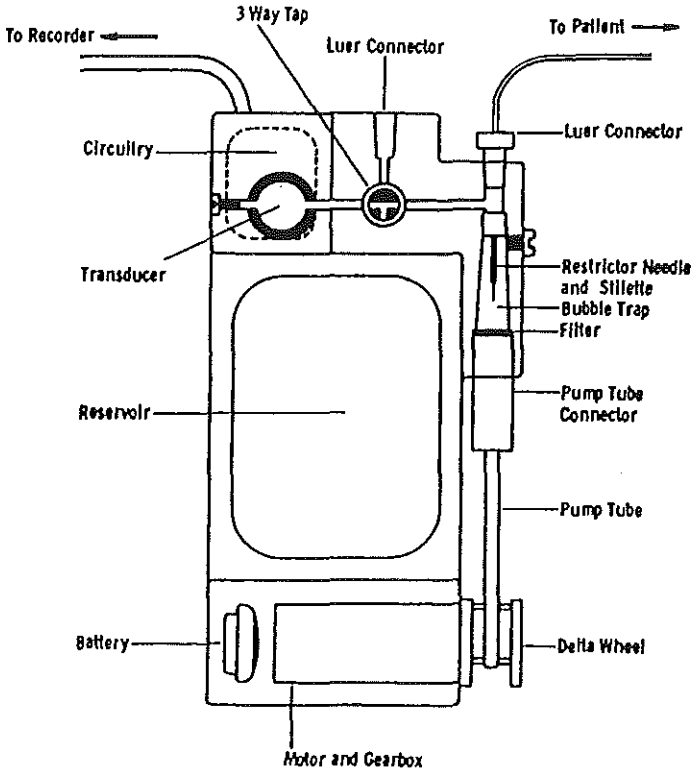

Figure 1. Transducer perfusion unit (reprinted from instruction manual)

saline solution via a hypodermic needle which is punctured through a rubber bung. Perfusion is maintained by a miniature peristaltic roller pump that delivers approximately 2.0 mL/h. The maximum pressure developed is 500 mmHg, which ensures that perfusion is independent of blood pressure. The pump motor is powered by a separate mercury button battery. A bubble trap prevents that air bubbles that may dissolve from the heparinized saline solution enter the catheter transducer system. A restrictor separates the catheter system from the large compliance of the pump and reservoir system. The transducer perfusion device weighs about 300 g.

Recording System

The blood pressure signal, after being converted to an electrical signal by the transducer, is recorded on an Oxford Medilog tape recorder (Medilog Recorder II, Oxford Medical Instruments, Oxford, England). To attain a stable recording sensitivity throughout the registration, the blood pressure signal is recorded on tape by pulse width modulation (Figure 2). For signal modulation a triangular carrier wave with a frequency of 40 Hz is used. The output voltage of the transducer is continuously compared with the carrier wave

voltage, and at the intersections of these two signals, pulses are generated and recorded on tape. A positive pulse is generated where the carrier wave becomes larger than the signal, and a negative pulse is generated at the intersection where the carrier wave becomes smaller than the signal. The time interval t_m between a negative pulse and the next positive pulse is linearly related to the voltage. In order to correct for small variations in tape speed, t_m is divided by the distance between two negative pulses t_c. Tape speed is maintained by phase-locked motor speed control that reduces speed variations to a maximum of 2%. The recorder weighs approximately 400 g.

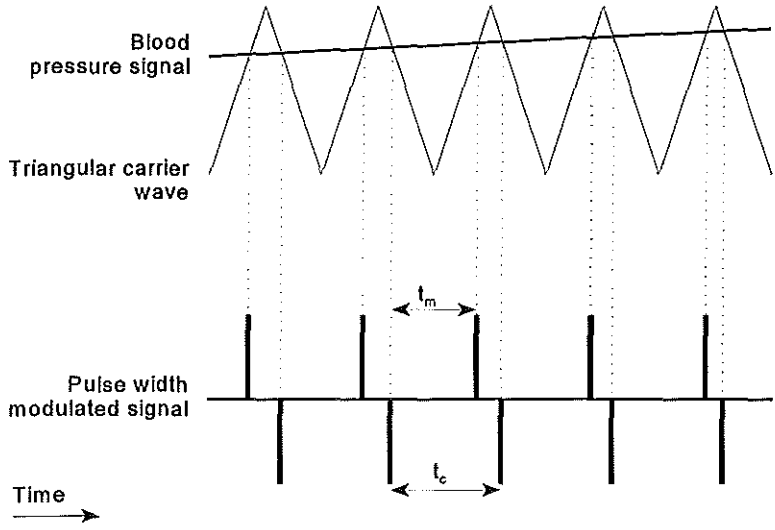

Figure 2. Pulse width modulation. The pressure signal voltage from the transducer, here depicted as linearly increasing, is compared to a 40 Hz carrier wave voltage. At intersections of the two waveforms, positive (carrier voltage increases over signal voltage) and negative (carrier voltage decreases below signal voltage) pulses are recorded on tape. Ratios of pulse interval times t_m/t_c are related to transducer voltage and therefore to blood pressure.

Modifications of the Original Recording System and Recording Procedure

In their first blood pressure recordings, van Montfrans and co-workers found a considerable drift of 5-15 mmHg during the first hours of the registration. This phenomenon appeared to be caused by a dependency of the transducer recording system on battery voltage[5] and on temperature[6]. Battery voltage decay induced both drift and a change of pressure sensitivity of the recording system. It occurred mainly during the first hours of the registration since voltage decay is most obvious during that period. Battery voltage induced drift ranged up to 10 mmHg in the physiological range of blood pressure

(50-250 mmHg) taking into account the normal battery decay during the registration. The problem was circumvented by the application of a voltage stabilizing circuit.
Temperature induced drift is predominantly caused by the transducer and its electronic circuit and amounts to approximately 0.5-1.0 mmHg/°C. If the transducer and recorder are at room temperature during the calibration procedure, a drift of approximately 10 mmHg is likely to occur within the first hours of a registration while the unit warms up to skin temperature. After that no sizeable drift is seen. To avoid temperature induced-drift, the transducer-recording system is pre-warmed to 30°C in a heat cabinet before calibration and recording. In order to maintain a stable temperature, the transducer-recording system is worn on the body in a padded pouch under the clothing, during the registration.
With these technical and procedural modifications, drift was reported to be smaller than 5 mmHg[4].

Recording Procedure

Before use, the reservoir, transducer and all connections were sterilized with glutaraldehyde 2% in 0.3 M $NaHCO_3$. The recording device and the transducer perfusion device were slowly pre-warmed to 30°C in a heat cabinet for at least 3 hours. Before the start of the registration procedure, a calibration pressure sequence of 50, 150 and of 200 mmHg was recorded. Pressure was applied with a hand-inflatable air manometer system (Amtek Texim 5093 PM, Tradinco Instrumenten Apparaten B.V., Berkel en Rodenrijs, The Netherlands). Each pressure level was maintained for at least one minute.
The subjects who participated in the studies were instrumented in the cardiovascular research laboratory of the Department of Internal Medicine 1 of the University Hospital Dijkzigt. After undressing to the waist they lay recumbent with the non-dominant arm extended and exo-rotated. After palpation of the brachial artery, the skin was sterilized with chlorohexidine 0.5% in ethanol 70%. The skin and subcutis were anaesthesized with a 2% lidocaine solution, and a Teflon catheter was introduced in the brachial artery by means of the Seldinger technique, 1-2 cm above the antecubital crease. After cannulation of the brachial artery the cannula was connected to a manometer extension tube, and the cannula and the extension tube were flushed with saline, carefully removing all trapped air. The perfusion transducer unit and recorder were fitted at heart level in a padded harness. After stripping of the extension tube to the skin, the elbow was loosely bandaged. Subsequently the clothes were fitted again, leaving the transducing perfusion unit and the recorder under the clothes. After it was confirmed that the quality of the blood pressure wave was sufficient by visualizing the signal on a monitor, the blood pressure registration was started.
At the end of the day the subjects were seen again. During this visit, the perfusion reservoir of the catheter manometer system was filled, and a second calibration procedure was performed. At the end of the registration on the next day, a third calibration sequence was recorded after which the cannula was removed. A pressure bandage was applied to the puncture site which was left in place at least 12 hours.
Each registration was documented with the personal details of the subject, the date and time of the start and the end of the registration, and the time and pressure levels of the calibration sequence recording.

Safety of Intra-Arterial Blood Pressure Recording

Arterial cannulation is associated with a documented appreciable morbidity[8]. However, most centers routinely performing intra-arterial measurement of blood pressure have reported that significant complications are exceedingly rare. The Northwick Park group[9] in a clinical audit of 1000 cases of intra-arterial cannulation reported one major complication: an infected pseudoaneurysm of the brachial artery[10]. Minor complications reported where local hematoma (4-5%), hemorrhage (1-2%), discomfort at the insertion site (3%), and vaso-vagal reaction at insertion of cannula (1-2%). Median nerve compression by hematoma formation has also been reported with the Oxford technique[11]. Other recognized complications of prolonged radial artery cannulation are radial artery thrombosis, embolic phenomena, bacteremia and septic complications. In a review of the first 600 intra-arterial measurements performed in our centre by means of the Oxford technique, we found short-lasting paresthesias of the finger tips (<1%), transient white or blue discolorations of the fingers (<1%), and hematomas at the puncture site (<1%). Infections, arterial occlusion, or nerve damage did not occur.

Replay and Data Aquisition

The recordings were replayed at 60-fold speed using an Oxford PMD 12 or Oxford PB-2 replay unit (Oxford Medical Instruments, Oxford, England). In order to obtain an overall impression of signal quality, the pressure signal was written on paper by means of a chart recorder (TA2000, Gould Inc., Cleveland, Ohio, USA). For further analysis, the analog signal from the replay unit was converted to digital values with a quantification precision of 12-bits (0 to 4095) by an AT CODAS AD-convertor (Dataq Instruments Inc. Abron, Ohio, USA) installed in a personal computer (Olivetti XP/7, Olivetti, Ivrea, Italy). The sensitivity of the AD-convertor was adjusted to the output voltage of the replay unit using a DI-420 pre-amplification unit (Dataq Instruments Inc.). The signal was sampled with a frequency of 10 kHz, corresponding to 167 Hz real time. The digitized signal was stored on the magnetic disk of the computer system for later analysis (see Chapter 3).

Technical Characteristics

The technical specifications of the blood pressure recording system are published by the manufacturer[2]. A more thorough evaluation of the system can be found in the thesis of van Montfrans[4]. The most important limitations of sytem as described by these authors are a limited frequency response, and a temperature induced drift up to 5 mmHg.
We measured the technical characteristics of the combination of transducer, recorder and replay system using the calibration sequences of 22 unselected blood pressure recordings. This has the advantage that the performance is assessed as it is in daily practice instead of under controlled evaluation conditions. In this way, the linearity, drift, and signal to

noise ratio of the complete measuring system were assessed. The tape speed stability, the signal to noise ratio and the frequency response of the recorder were measured by means of artificial signals.

Linearity

Linearity of the recorder transducer system was assessed as the difference of the measured 150 mmHg calibration pressure level and the level that was calculated by interpolation between the 50 mmHg and the 200 mmHg pressure levels. The results are listed in Table 1. The mean±SD of the average deviation was 0.09±0.38 mmHg (range, -0.65 to 0.95 mmHg).

Signal to Noise Ratio

For the measurement of the signal to noise ratio (SN-ratio) of the whole system, the standard deviation of the signal during the application of calibration pressures was calculated. The standard deviation appeared to be independent of pressure and ranged from 1.9 mmHg to 2.8 mmHg (SN-ratio 30 dB to 32 dB).

For the measurement of the SN-ratio of the recorder and replay system alone, constant voltage levels corresponding to 50 mmHg, 150 mmHg, and 200 mmHg were applied to the recorder for 60 seconds. The standard deviation of the recorded signal ranged from 1.2 mmHg to 1.6 mmHg, which corresponds to approximately 40% to 50% of the standard deviation of the whole system. The corresponding SN-ratio of the recoder and replay system is 36 dB to 38 dB, which is in accordance with the specification of the manufacturer (better than 30 dB).

Drift

Three recordings could not be used for the assessment of drift since in these only one calibration sequence was recorded. In the remaining 19 recordings, drift was measured as the maximum deviation of the second and third calibration line

Table 1. Drift and Deviation from Linearity Calculated from Calibration Sequences in Unselected Blood Pressure Registrations

Registration	Deviation from Linearity (mmHg)	Drift at 50 mmHg (mmHg)	Drift at 200 mmHg (mmHg)
1	0.23	3.5	2.2
2	0.33	7.5	6.9
3	0.10	-0.1	1.5
4	0.42	4.5	2.4
5	-0.08	-3.7	-0.4
6	0.06	4.1	4.2
7	0.07	5.8	3.9
8	0.95	0.9	-0.4
9	0.82	7.0	8.3
10	-0.65	5.4	5.0
11	-0.03	3.7	2.5
12	-0.42	3.7	3.6
13	0.28	7.4	8.0
14	0.19	4.2	4.6
15	-0.09	2.8	4.0
16	-0.26	3.2	6.6
17	-0.10	6.8	4.6
18	-0.24	3.6	5.1
19	0.15	1.0	5.0
20	0.46	.	.
21	-0.33	.	.
22	0.19	.	.
Mean±SD	0.09±0.38	3.8±2.8	4.1±2.4

from the first calibration line at 50 mmHg and at 200 mmHg. The results are shown in Table 1. Drift was independent of pressure level and amounted to 3.8±2.8 mmHg at a pressure level of 50 mmHg (range, -3.7 mmHg to 7.5 mmHg) and to 4.1±2.4 mmHg at a pressure level of 200 mmHg. In eight registrations, drift was larger than 5.0 mmHg. The drift occurred mainly between the first and second calibration (data not shown).

Frequency Response

According to the manufacturer[2], the frequency response of the whole blood pressure measurement system including the catheter is flat up to 8 Hz (-3 dB) and decreases sharply at higher frequencies. The frequency response is limited by the 40 Hz pulse width modulation sampling of the recorder and, more importantly, by a 10 Hz low-pass filter that is part of the replay unit and serves to remove carrier wave effects from the signal[7]. The limited frequency response of the recorder, but also the attenuation and reflection of the pressure wave by the catheter transducer system may cause distortions and blunting of the arterial pressure wave form as it is recorded on tape. These effects were assessed for systolic and diastolic pressure by Millar-Craig[2] who measured blood pressure directly in the aorta of a dog by means of a catheter tip transducer and compared it to the blood pressure that was measured simultaneously by an Oxford Medilog system. He found an underestimation of systolic and diastolic pressure which was on average 3% and always smaller than 5 mmHg in the range from 90 mmHg to 190 mmHg.

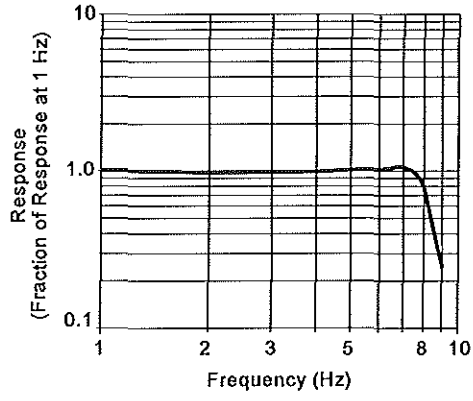

Figure 3. Frequency response of the Medilog II recorder (recorder A)

We verified the frequency response of the recorder by recording electronically generated sine waves of increasing frequencies. The frequency response (see Figure 3 for an example) was found to be identical to the specifications of the manufacturer, i.e. flat up to 8 Hz (-3 dB).

Tape Speed Stability

Short term tape speed stability was assessed by recording a 1 Hz saw-tooth wave during 150 seconds. After replay and AD-conversion of the signal, the coefficient of variation of the distances between the saw-tooth tops was calculated. The short term tape speed variation ranged from 1.3% to 1.5%. Tape speed stability during the day was assessed by comparing the actual time of the second calibration procedure with the measured time. The deviation was never more than 20 minutes, i.e. 1.4% of 24 hours.

Discussion

The deviation from linearity in our measuring system is small (0.09±0.38 mmHg, range, -0.65 to 0.95 mmHg). The quantitative effect of the deviation from linearity in our studies will be even smaller, since the blood pressure signal was calibrated by interpolation

between all calibration levels instead of by interpolating between the lowest and highest calibration levels (see Chapter 3).
The noise that we measured in the Oxford Medilog system will add some variation to hemodynamic parameters. The addidtional variation will be most prominent in parameters that are based on a single pressure measurement, e.g., the systolic pressure. In the experiments for the evaluation of the blood pressure analysis program (see Chapter 3) we measured a standard deviation of the systolic pressure over 36 s periods that was in the range from 2.0 mmHg to 10.0 mmHg with an average value of 6.3 mmHg. If these values are corrected for the standard deviation of the measurement system, assuming a value of 1.5 mmHg, the true standard deviation of systolic pressure will be on average 0.2 mmHg smaller than the measured standard deviation (range, 0.7 mmHg to 0.1 mmHg). The standard deviation added by the measurement system to the standard deviation of parameters that are measured over a number of samples will be considerably smaller, e.g., for the mean arterial pressure typically less than 0.02 mmHg. The contribution of the measurement system to the uncertainty of parameters that are calculated over time periods longer than one minute is negligible, because of the large number of measurements involved and the considerable long term intrinsic blood pressure variation.
A more important source of error is the drift of the recording system which influences all measured pressures. The drift amounted on average to 4 mmHg and occurred despite the inclusion of a voltage stabilizing circuit and pre-warming of the recorder and transducer unit. Most of the drift occurred between the first and the second calibration, and, probably, during the first hour of the registration since the most important cause of drift is an adaptation of the measuring system to skin temperature[4]. We did not assess the exact pattern of drift during the registration, and corrected the effect of drift by linear interpolation between the recorded calibration sequences. The exact merits of this correction procedure are unknown. However, assuming that the sign of the drift does not change between two calibration sequences, a compensation for drift by means of linear interpolation will decrease the maximal average error over the time period by 50%. Assuming this model and assuming that drift only occurs between the first and the second calibration on the first day of the registration (typically at 8:00 hour and at 16:00 hour), the maximal error of 24-hour mean arterial pressure is 0.8±0.5 mmHg, and the maximal error in the nocturnal change of mean arterial pressure is 1.8±1.3 mmHg. Differences between registrations in the same individual, or between individuals will not be biased by drift if both groups are measured under the same conditions.

Conclusion
We found that drift and wave form distortion are the most important sources of error for measuring blood pressure with the Oxford Medilog system. Although we did not assess the quantitative effect of the drift on the measurement of 24-hour blood pressure, the effect is probably less than 0.8±0.5 mmHg for the 24-hour average blood pressure, and less than 1.8±1.3 mmHg for the nocturnal change in blood pressure. The bias will be much smaller if the study outcome is the difference between blood pressure registrations recorded under similar conditions.
We did not repeat the measurements of the effects of wave form distortion of the blood pressure curve performend by Millar-Craig[2], since most of our studies are based on the measurement of mean arterial pressure, which is insensitive to wave form distortion.

However, in Chapter 10 we assessed stroke volume from the pressure curve by a pulse contour method. Blunting of the arterial waveform makes the location of the dicrotic notch more difficult to detect. This detection of the dicrotic notch will be evaluated in Chapter 3. The blunting of the pulse waveform also induces a systematic error in the estimation of the stroke volume. The quantitative effect of this systematic error is limited by the fact that we only calculated relative changes in the stroke volume within the same individuals. The magnitude of this error, however, remains unknown.

References

1. Littler WA, Honour AJ, Sleight P, Stott FD: Continuous recording of direct arterial pressure and electrocardiogram in unrestricted man. Br Med J 1972;3:76-78
2. Millar-Craig MW, Hawes D, Whittington J: New system for recording ambulatory blood pressure in man. Med Biol Eng Comput 1978;16:727-731
3. Mancia G, Ferrari A, Gregorini L, Parati G, Pomidossi G, Bertinieri G, Grassi G, Zanchetti A: Blood pressure variability in man: Its relation to high blood pressure, age, and baroreflex sensitivity. Clin Sci 1980;59:401S-410S
4. Van Montfrans GA: Continuous ambulatory blood pressure registration in uncomplicated hypertension. Cuff-responders and blood pressure variability. Amsterdam: University of Amsterdam, 1984. Dissertation.
5. Grimbergen CA, van Montfrans GA, Pos M, Borst C, Dunning AJ: Battery voltage dependence of the Oxford Medilog blood pressure system. In: Stott FD, Raftery EB, Clement DL, Wright SL. eds. Proceedings of the fourth international symposium on ambulatory monitoring and the second Gent workshop on blood pressure variability. London: Academic Press 1982, 438-443
6. Grimbergen CA, van Montfrans GA, Pos M, Borst C, Dunning AJ: Temperature dependence of the Oxford Medilog blood pressure measuring amplifier. In: Stott FD, Raftery EB, Clement DL, Wright SL. eds. Proceedings of the fourth international symposium on ambulatory monitoring and the second Gent workshop on blood pressure variability. London: Academic Press 1982, 433-437
7. Cashman PMM: How shall we analyse the ambulatory blood pressure signal? In: Marchesi C, ed. Ambulatory monitoring. Cardiovasular system and allied applications. Hingham, USA: Martinus Nijhoff Publishers, 1984, 252-268
8. Morternsen JD: Clinical sequelae from arterial needle puncture, cannulation and incision. Circulation 1967;35:1118-1123
9. Mann S, Jones R, Millar-Craig M, Wood G, Gould B, Raftery EB: The safety of ambulatory intra-arterial pressure monitoring, a clinical audit of 1000 studies. Int J Cardiol 1984:5:585-597
10. Wagner M: Pseudoaneurysm. A complication of percutaneous angiography and angiocardiography. J Am Med Assoc 163;186427-428
11. Littler WA: Median nerve palsy - a complication of brachial artery cannulation. Postgrad Med J 1979;52(suppl 7):110-111

Chapter 3

Analysis of the Blood Pressure Signal

Beat Parameters

During the contraction phase of the heart, the systole, blood is expelled from the left ventricle and forced into the aorta. This results in a sharp increase of aortic blood pressure. At the end of the contraction phase, aorta pressure decreases rapidly until the aortic valve closes. The ventricle relaxation phase, the diastole, is characterized by a gradual decrease of blood pressure as blood flows to peripheral vessels against the vascular resistance. Frequently the diastole starts with a small increase of pressure, the dicrotic notch, resulting from the abrupt closing of the aortic valve. The rhythmic expulsion of blood from the heart, the action of the aortic valve and the hemodynamic characteristics of the arterial tree determine the shape of the blood pressure curve. During the propagation of the pressure pulse along the vascular system, the contour of the blood pressure wave gradually changes, resulting in an increase of systolic pressure and a decrease of mean arterial pressure. This phenomenon is caused by wave distortion due to the inhomogeneous structure of the arterial tree, and by the vascular resistance.

Figure 1 demonstrates an example of the blood pressure curve in the brachial artery. Various phases of the heart action can be recognized in the pressure curve.

The beginning of a beat is defined by a steep rise of blood pressure. This steep ascent, called the *upstroke*, reaches from the end-diastolic pressure (EDP) to a maximum called the systolic pressure (SP). Note that the end-diastolic pressure level is not determined by the current beat, but instead by the previous beat. The pressure increase during the upstroke is called pulse pressure. After the systolic pressure, blood pressure decreases rapidly. Following the closure of the aortic valve, recognizable as the dicrotic notch (DN), the blood pressure decreases more slowly and with some variations to the end-diastolic pressure that marks the beginning of the next upstroke. The minimum pressure during this phase is called the diastolic pressure (DP). The period between the systolic pressure and the subsequent end-diastolic pressure is referred to as the downstroke. The duration of the beat, the period between the start of two successive upstrokes, is called the interbeat interval (II) or beat length. The instantaneous heart rate (IHR) is calculated from the interbeat interval according to (1). In this formula, IHR is expressed in beats per minute (bpm) and interbeat interval in seconds.

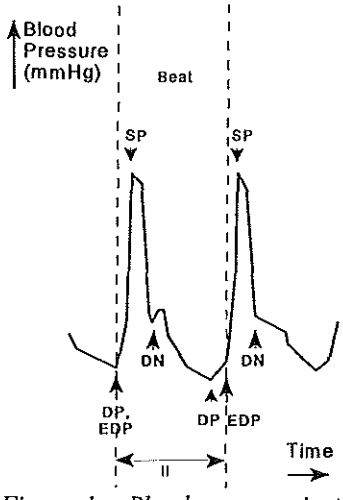

Figure 1. Blood pressure in the brachial artery as a function of time. EDP indicates end-diastolic pressure; SP, systolic pressure; DP, diastolic pressure; DN, dicrotic notch; II, interbeat interval or beat length.

$$IHR = \frac{60}{II} \qquad (1)$$

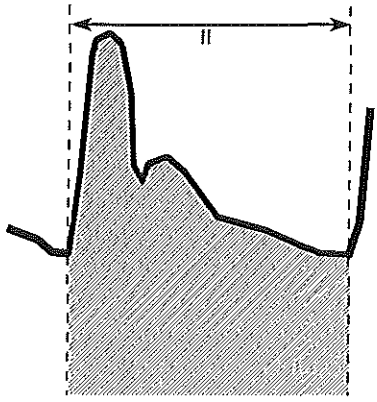

Figure 2. Integrated mean arterial pressure is calculated as the area under the pressure curve (hatched) divided by the interbeat interval II.

The integrated mean arterial pressure (MAP) is defined as the true average pressure during the beat, i.e., the area under the pressure curve of the beat divided by the interbeat interval (Figure 2).

Estimation of Stroke Volume, Cardiac Output and Total Peripheral Resistance from the Pulse Contour

Stroke volume was assessed by a corrected pulse-contour method developed by Smith et al[1,2]. The calculation of stroke volume from the pulse contour is based on a simple relation between flow and pressure in time[3]. The total amount of blood that is ejected in the aorta during the systole depends on the *specific impedance* to flow in the aorta, Z_{ao}, and on the driving force, i.e., the product of the increase in blood pressure during the systole and the length of the systole. This product corresponds to the pulsatile systolic area (PSA), which is delimited by the level of end-diastolic pressure at the start of the beat, the blood pressure curve and the time of the dicrotic notch (Figure 3).

The relation between flow and pressure in time is expressed in (2).

$$SV = \frac{PSA}{Z_{ao}} \qquad (2)$$

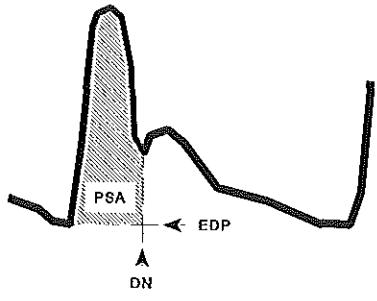

Figure 3. The pulsatile systolic area PSA is delimited by the end-diastolic pressure level EDP, the dicrotic notch DN and the pressure curve.

PSA can be obtained from the blood pressure signal directly. The value of Z_{ao} appears to be dependent on the instantaneous hemodynamic condition of a subject. Wesseling et al[2] investigated this dependency of Z_{ao} on blood pressure, heart rate, and on total peripheral resistance.

With increasing blood pressure, the cross-section of the aorta increases and the compliance decreases in an age-dependent way. The net effect of these phenomena is that Z_{ao} increases with mean arterial blood pressure.

The value Z_{ao} decreases with heart rate. This can be explained by the increase of impedance of the arterial tree towards the periphery. The longer the duration of the ejection period is, the more impedance the pressure wave will meet on its way. The

value of Z_{ao} will therefore increase with beat length, and decrease with heart rate. The dependency of Z_{ao} on heart rate is strongest in arteries with a low compliance, and is independent of total peripheral vascular resistance.
Based on these findings, Wesseling determined correction factors for mean arterial pressure and heart rate (3).

$$SV = \frac{PSA * (a + b*IHR + c*MAP)}{d} \quad (3)$$

The values of the correction factors *a*, *b*, *c*, and *d* are age-dependent and are provided by Stok[4] and Wesseling[5].
Although on average the correction factors are appropriate, the exact value of Z_{ao} for the individual subject is still unknown, and in order to calculate true values for stroke volume, the corrected pulse contour method must be calibrated for individual subjects against a method that measures an absolute value, e.g. a dye-dilution method or an echocardiographic technique. If such a calibration is not available, however, the corrected pulse contour method can still be used to calculate relative changes of stroke volume.
The stroke volume is used to calculate the instantaneous values of cardiac output and total peripheral resistance, i.e., the values of these parameters during the current beat. These values are later used to calculate, e.g., 20-minute averages of these parameters, weighing the instantaneous values with the corresponding beat lengths. Instantaneous cardiac output ICO is calculated from heart rate and stroke volume (4).

$$ICO = SV * IHR \quad (4)$$

Instantaneous total peripheral resistance ITPR is estimated from mean arterial pressure and cardiac output, assuming a right atrial pressure of 0 mmHg (5).

$$ITPR = \frac{MAP}{ICO} \quad (5)$$

Since stroke volume was not calibrated against a true value, in our studies stroke volume, cardiac output and total peripheral resistance are expressed only as percentages of their 24-hour averages, which were set at 100%.
The accuracy of cardiac output measurement by means of this method has been established by comparison of cardiac output estimated by the pulse-contour method with cardiac output simultaneously estimated by thermodilution in seven patients undergoing a coronary bypass graft operation[6]. The two methods were well correlated ($r=0.94$; $n=64$) over the range of cardiac output values that were measured during surgery(2.5 to 8.0 L/min). The standard deviation of the differences between the methods expressed as the mean of the methods was 10.6%. The line of linear regression between the methods

was $CO_{pc}=0.3+0.94*CO_{th}$, in which CO_{pc} and CO_{th} are the cardiac output estimated by the pulse contour method and by thermodilution, respectively. This implies a small, cardiac output dependent bias of the pulse contour method with respect to the thermodiluation method. The bias is probably somewhat smaller than calculated by the authors. The standard deviation of the measurement repeatability is 7% for the thermodilution method and 2.5% for the pulse contour method[6]. Since neither of the two parameters can be considered to be an independent variable, the correct method for calculating the regression line is by means of weighted orthogonal regression[7]. Whereas linear regression minimizes the square error of the Y-variable, orthogonal regression minimizes the weighted combined square error of both variables and therefore results in a slightly larger regression slope, in particular when the variable with the largest standard deviation is incorrectly used as the independent variable in the calculations for linear regression. A more fundamental limitation of the comparison between the two methods which is mentioned by the authors[6] is that in fact there is no golden standard to which the measurement of cardiac output can be compared. Although estimation of the cardiac output by the thermodilution technique is well accepted, the assumptions that underly its use may not allways be fulfilled[6]. Therefore, it cannot be excluded that the differences between the methods are caused in part by the thermodilution method. Assuming, however, that the thermodilution measures cardiac output correctly and that the regression equation represents the error of the pulse contour method, the bias in the calculation of the pulse contour can be estimated. For values of cardiac output during the day of 2.5, 5, and 10 L/min, and a nocturnal decrease in cardiac output of 20%, the nocturnal changes as calculated by means of the pulse contour method are 17.7%, 18.8%, and 19.4% respectively, indicating a small underestimation of the nocturnal decrease in cardiac output by the pulse contour method.

Calibration and Editing

For the calibration of the digitized signal the program PRE-ANALYSIS was developed (Turbo Pascal 6.0, Borland International Inc., Scotts Valley, California, USA). This program allows the investigator to inspect the signal in a graphical form on a computer display. The investigator uses the program to specify the date and time of the start and the end of the registration and the calibration pressures applied with each calibration level. In addition, the investigator may exclude parts of the signal from analysis. The user interface of the program employs a pointing device (*mouse*).

Calibration of Time

A linear relationship is assumed between the sample sequence number and the sample time. The scaling parameters are calculated by linear interpolation from the times of the start and the end of the registration and the number of samples that has been recorded during this period. The date and time of an event in the recording are calculated from the sample sequence number of the first sample of the event.

Calibration of Pressure

The PRE-ANALYSIS program employs three calibration sequences in the registration. The calibration sequences are recorded at the beginning of the registration, after approximately 8 hours, and at the end of the registration. Each calibration sequence

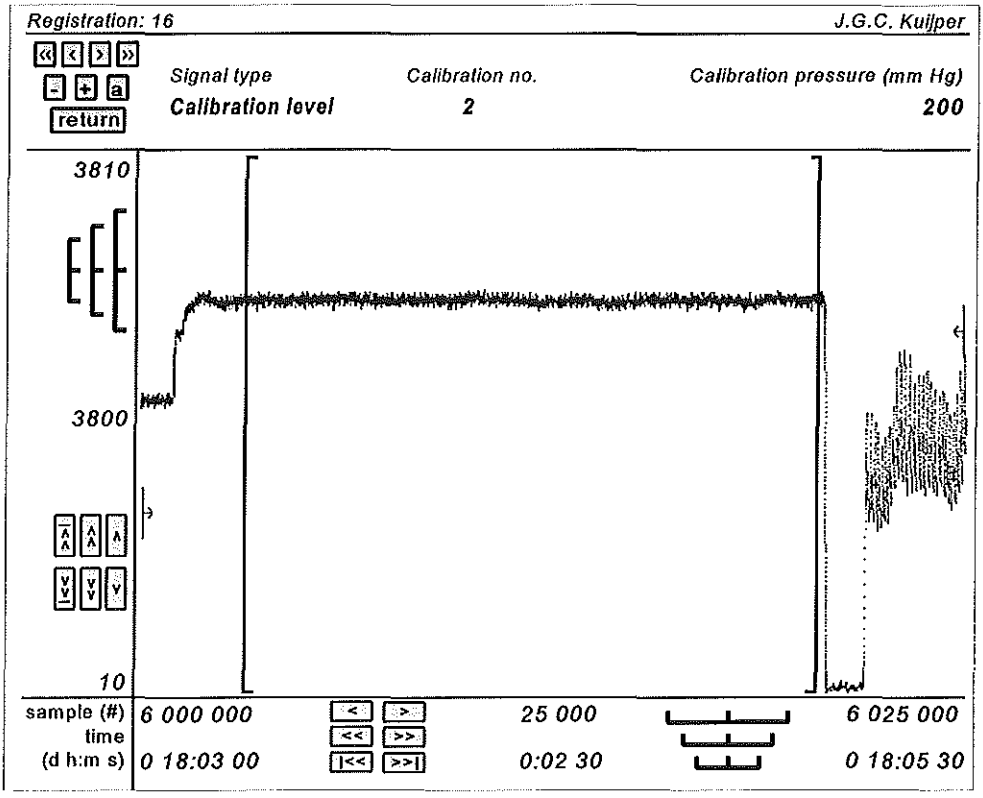

Figure 4. Schematic representation of the display of the calibration and signal editing program. The last calibration level (200 mmHg) of the second calibration sequence is displayed. Data in italics can be modified on the screen for the specification of calibration pressures (upper panel) or modification of the range of the pressure axis (left) and time axis (bottom). Button symbols on both axes allow horizontal and vertical navigation through the signal and scale modification of the window by single mouse clicks. Subject name is fictitious.

consists of three consecutive pressure levels of 50 mmHg, 150 mmHg and 200 mmHg, each with a duration of at least 60 s. The calibration is calculated by linear interpolation between consecutive calibration levels, and by linear extrapolation for pressures smaller than 50 mmHg or larger than 200 mmHg. Drift occurring between calibration sequences is compensated by linear interpolation between the calibration sequences.

The program detects the calibration sequences and subsequently displays the calibration pressure levels, delimited by square brackets, for visual control (Figure 4). The investigator specifies the pressure corresponding to each of the calibration levels. Though the specification of calibration pressures can be completely automated, this is not

implemented. The investigator is confronted with the calibration sequences as they are recorded, as a visual check of the signal quality.

Excluding Signal Fragments from Analysis by Observer Verification
At this stage of the editing procedure the investigator can inspect all parts of the registration and specify intervals that are to be excluded from the analysis. The investigator specifies a signal fragment by superposing a pair of markers on the signal (Figure 5) and by indicating that the signal fragment between these markers is an artifact. In the present studies, no signal fragments were excluded from analysis by this procedure. Both the time scale and the pressure scale of the display can be changed, allowing the investigator, e.g., to show in detail a pressure interval that is of interest or to display an overview of a time period that is of interest. The ranges of the axes can be changed to specific values by directly specifying the minimum, the maximum or the range of an axis. The axes can also be modified by activating button symbols for scaling

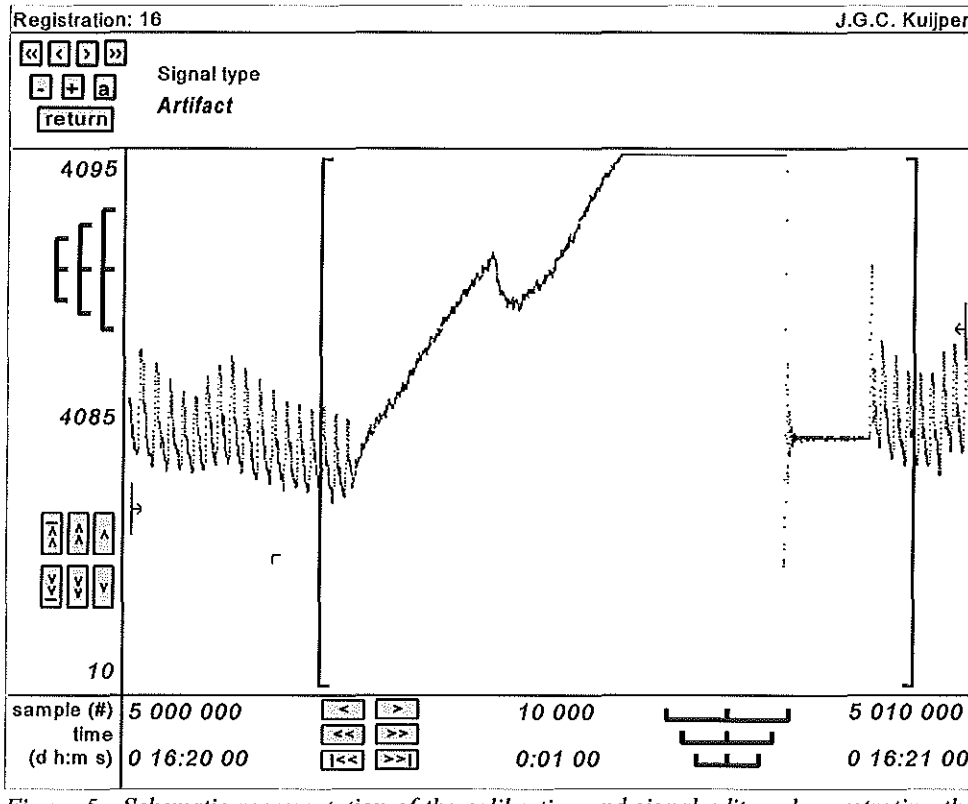

Figure 5. Schematic representation of the calibration and signal editor, demonstrating the exclusion of signal from beat to beat analysis. A blood sample was drawn through the intra-arterial catheter on the first recording day at 16:20 hour. During blood sampling the manometer tube was obstructed, and the pressure in the transducer system increased by the action of the flushing pump. The signal fragment is marked by a pair of square brackets. These are added to the signal by clicking the "+" button (above, left) and subsequently dragged by means of the computer mouse to the positions that delimit the intended signal fragment. Subject name is fictitious.

(outer ⊢⊣ symbols) and translation (e.g. ⊠) that are depicted beside each axis. This allows the investigator to zoom in and zoom out or to scroll throught the signal, both in the horizontal and vertical direction, by single mouse clicks. Both axes are provided with a symbol (middle ⊢⊣ symbol) for the restoration of the original zoom factor.

Beat to Beat Analysis

Introduction

The process of the detection of individual beats in the digitized blood pressure registration and the calculation of the beat parameters is called beat to beat analysis. The computer program ANALYSIS was developed to perform this analysis (Turbo Pascal 6.0, Borland International Inc., Scotts Valley, California, USA). The result of the beat to beat analysis is a file containing calibrated, time-stamped parameters of all beats detected in the registration. This file serves as a basis for further calculations, e.g., calculation of the 24-hour average.

In all segments of the signal classified as blood pressure curve, the analysis program detects individual beats and calculates the beat parameters. This process is described in detail below. In short, the characteristic contour of a beat in the brachial artery is detected by searching for a rapid increase and subsequent decrease of blood pressure (*peak*), immediately followed by a more gradual decrease of pressure that is terminated by a pressure increase (*trough*). A peak-trough combination is considered to be a *candidate beat* (see *Detection of Candidate Beats*). Since the end-diastolic pressure at the beginning of a beat is determined by the preceding beat, and the beat length is determined by the start of the next beat, valid beat parameters can be calculated only if a beat is immediately preceded and followed by other beats. Therefore, the program rejects the first and the last candidate beat of a series. Candidate beats are considered to be part of a series if the distance between the starting points of the peaks is at most 3.0 s. Later during the analysis procedure, more stringent criteria are applied to beat length (see *Additional Criteria*). After detection of a candidate beat, the program tests the upstrokes of the candidate beat and the next candidate beat for continuous pressure increase (see *Upstroke Criteria and Feature Extraction*). During this procedure, the times of systolic pressure, diastolic pressure and end-diastolic pressure are determined. If both upstrokes are valid, the start and the end of the candidate beat can be determined. This allows testing of the candidate beat using additional criteria (see *Additional Criteria*). If a candidate beat is rejected, it is not considered as a reliable delimiter of the next candidate beat, in particular since the end-diastolic pressure may not be estimated correctly. Therefore, the next candidate beat is rejected as well. If all tests are passed, beat parameters are calibrated to mmHg and seconds and are stored in a file on computer disk.

Detection of Candidate Beats

The signal classified by the investigator as blood pressure signal is searched for a rapid increase and subsequent decrease of blood pressure (*peak*), immediately followed by a more gradual decrease of pressure that is of longer duration and terminated by a pressure increase (*trough*). For this purpose, simple and computationally efficient detectors are used that are most sensitive to the speed of pressure change during the peak and trough.

Since the width of the peak and trough are proportional to the beat length, the detectors are continuously adapted to heart frequency during the analysis. Small peaks and troughs are not detected by the application of a threshold for detection.
Let B(t) be the function that describes the blood pressure signal as a function of time t, and let $\underline{ii}$ be the average beat length over the last 10 beats. The detection of peaks and troughs in the blood pressure signal is implemented by a filter

$$Y(t) = B(t+w) - B(t-w) ,$$

with w=0.1*$\underline{ii}$ s for the peak detector and w=0.2*$\underline{ii}$ s for the trough detector (Figure 9). In words, this filter calculates the pressure difference in the blood pressure signal over a period of 0.2*$\underline{ii}$ s for the detection of a peak, and over a period of 0.4*$\underline{ii}$ s for the detection of a trough. The transfer function[8] of $Y(t)$ is

$$G(f) = 2j \sin(2\pi w f) .$$

The filter induces a phase shift of $\pi/2$. The amplitude spectrum, depicted in Figure 6, is

$$A(f) = 2 \left| \sin(2\pi w f) \right| .$$

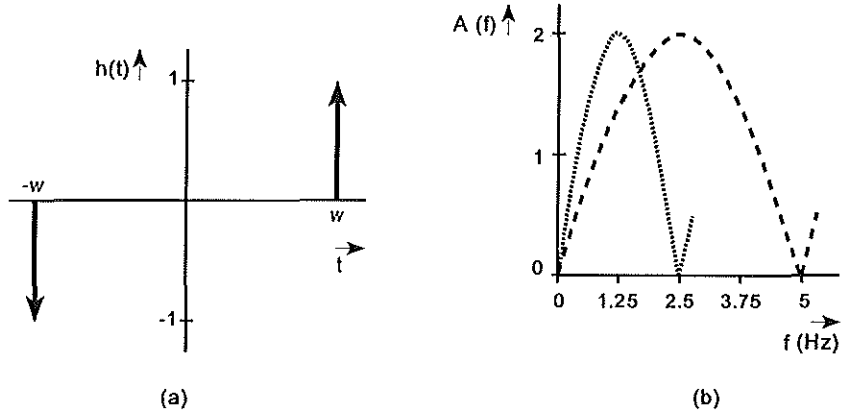

Figure 6. (a) Impulse response of Y(f). (b) Amplitude spectra of Y(f) for w = 0.1s (_ _, maximum at 2.5 Hz) and w = 0.2s (....., maximum at 1.25 Hz). These amplitude spectra correspond to the filter settings for the peak and trough detector at an average heart rate of 60 bpm over the last 10 beats ($\underline{ii}$ = 1.0 s). With these settings, the maximum sensitivity is at 2.5 Hz for peak detection and at 1.25 Hz for trough detection.

The filter is a simple band-pass filter. At a heart rate of 60 bpm ($\underline{ii}$ = 1.0 s), the bandwidth of the filter is between 1.2 and 3.8 Hz for the peak detector and between 0.6 and 1.9 Hz for the trough detector. In other words, the filter for peak detection is most sensitive to rapid pressure changes, and the filter for trough detection is sensitive to slower pressure changes.
A peak is detected as the filtered signal changes from positive to negative. This zero-crossing corresponds approximately to the maximum of the blood pressure signal, since the filtered signal is $\pi/2$ ahead of the original signal. In order to prevent small peaks from being detected, a threshold value of -102, corresponding to 5-10 mmHg, is applied to the filtered signal. Peak time is defined as the time of occurence of the detected peak.

A trough is detected at zero crossing of the filtered signal from negative to positive, applying a threshold of +102. Trough time is defined as the time of occurence of the detected trough. Trough detection commences at 0.2*ii s after peak time, and peak detection starts 0.2*ii s after trough time (see Figure 7).

A more intuitive description of the detector function is obtained by considering the detector as a first-derivate calculator. A peak, being a local maximum of the signal, is detected as the first derivative of the signal changes from positive to negative. The first derivative of the signal at a given time *t* is calculated as the pressure difference of the signal 0.1*ii s after *t* minus 0.1*ii s before *t*, divided by 0.2*ii s. However, since only the zero-crossing of the detector is of interest, the division by 0.2*ii s is omitted.

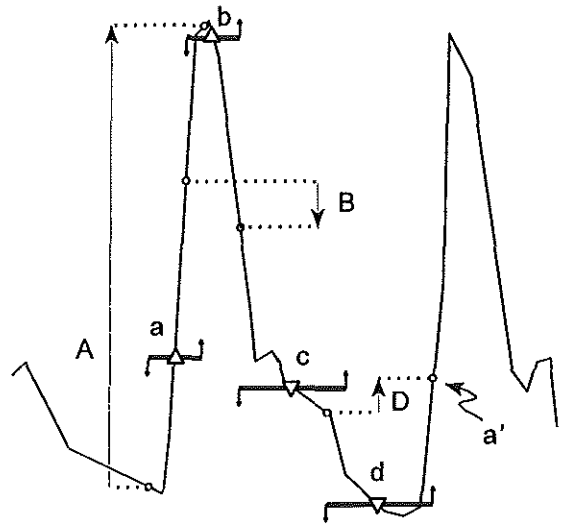

Figure 7. Detection of candidate beats. A peak detector is applied to the signal (a). The calculated value at (a) is positive (A) and the detector is moved along the signal until the position (b, peak time) where the output (B) is smaller than a negative threshold value. At this point a peak *is detected. Subsequently a trough detector is applied to the signal at point (c), 0.2*ii s after (b). The trough detector is moved along the signal until (d) (trough time), where the output (D) is larger than a threshold value. At this point, a* trough *is detected. Subsequently a peak detector is applied to the signal at (a'), 0.2*ii s after (d).*

Upstroke Criteria and Feature Extraction

The upstroke, the ascending part of a beat (Figure 7), is searched in the interval from 0.2*ii s before trough time to 0.1*ii s after peak time of the current candidate beat. First, the trough minimum pressure is located and marked as the diastolic pressure. Subsequently, the maximum pressure in the peak is detected and marked as the systolic pressure. When there are two or more minima or maxima having the same pressure level, the last minimum or the first maximum are marked as diastolic and systolic pressure, respectively. Subsequently, the maximum pressure ascent between the diastolic and systolic pressure is determined. The pressure ascent is calculated as the pressure increase in 25 ms (i.e. 4 samples). The program then searches backwards from the maximum pressure ascent until the pressure ascent is smaller than 0.1 of the maximal value or until a local minimum is detected, and marks this point as end-diastolic pressure. Subsequently the program verifies that pulse pressure is at least 1/40 of signal range, and that the pressure rise during the upstroke is not interrupted by a decrease of pressure before 80% of the pulse pressure level is reached.

Additional Criteria

In the following cases, a candidate beat is rejected:

Interbeat interval too short

The interbeat interval is shorter than 40% of the average interbeat interval over the 10 preceding beats. In the majority of cases such a short interbeat interval is caused by an

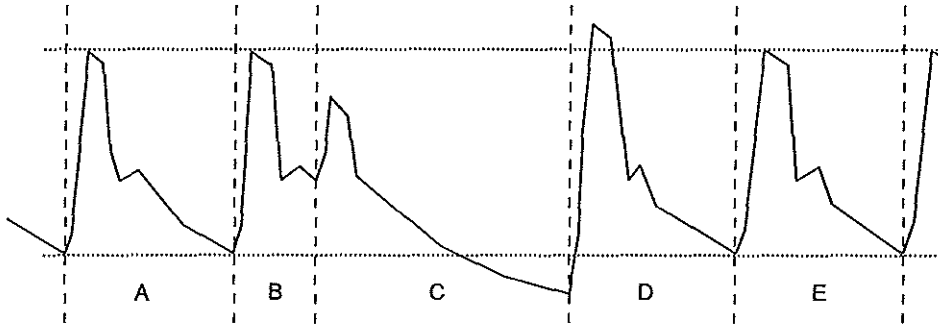

Figure 8. Rejection of candidate beats in case of detection of an extrasystole. Since the beat length of candidate beat B is shorter than 40% of the average beat length over the last 10 beats, candidate beat C is classified as an extrasystole, and candidate beats B and C are rejected. Since the extrasystole induces an increase of pulse pressure of candidate beat D that is not considered representative of normal blood pressure regulation, candidate beat D is rejected as well. In some cases, the pressure rise of beat C is small and will remain undetected. In such a case candidate beat B is likely to be rejected on the basis of an increased beat length. In this case, two candidate beats (B and D) instead of three will be rejected.

extrasystole. Therefore, the next two candidate beats are skipped, since the end-diastolic pressure of the current beat and the increased pulse pressure of the next beat are not considered to be representative of the normal blood pressure regulation (Figure 8). At the beginning of the analysis procedure, the average interbeat interval is set to 0.8 s.

Interbeat interval too long

The interbeat interval is longer than 170% of the average interbeat interval over the the 10 preceding beats, or longer than 1.6 s. At the beginning of the analysis procedure, the average interbeat interval is set to 0.8 s. This criterion may identify an extrasystole in which the pressure rise of the extra contraction is not detected (Figure 8).

Upstroke too long or too short

The duration of the upstroke is shorter than 10% or longer than 30% of the interbeat interval.

Path length criterion not met

The ratio path length upstroke / path length downstroke is smaller than 0.33 or larger than 1.2. The path length of the upstroke and downstroke are calculated by summing the absolute values of the pressure differences between the individual samples of the upstroke and the downstroke, respectively.

Detection of the Dicrotic Notch

The dicrotic notch is detected as the first local minimum after the systolic pressure. Detection is based on the output of a simple combined band-pass and first derivative filter. If no local minimum is found, a second derivative filter is used to detect the maximal decrease of the first derivate of the signal. The filter parameters were adjusted to each individual registration by visual inspection of the detection results.

Construction of 24-Hour Profiles

In the present studies, average values of hemodynamic parameters were calculated over periods of 3 minutes and over periods of 20 minutes. In order to calculate the integrated average values of mean arterial pressure, cardiac output and total peripheral resistance over these periods, the instantenous (beat to beat) values of these parameters were weighted with the duration of the beat. Since stroke volume, systolic pressure, diastolic pressure, end-diastolic pressure, heart rate and interbeat interval were considered as time point values, these parameters were averaged without a weighing factor. The averages and standard deviations over 24-hour periods of all parameters were calculated from the averages over 3 minute periods or 20 minute periods, respectively.

Since the pulse contour method does not allow calculation of the absolute values of stroke volume, cardiac output and total peripheral resistance, these parameters were expressed as a percentage of their 24-hour average value, which was set at 100%.

Evaluation of the Analysis

Outline of the Evaluation Procedure

The performance of the algorithm used for blood pressure analysis was quantified by comparing results of the ANALYSIS algorithm to *reference beats* obtained by visual inspection. A graphical editor EDIT was developed to generate such reference values (Turbo Pascal 6.0, Borland International Inc., Scotts Valley, California, USA). This editor displayed the blood pressure signal with superimposed results of beat detection by ANALYSIS, and allowed correction of the results of the blood pressure analysis program by visual inspection of the signal.

For the evaluation of ANALYSIS, the sensitivity and specificity of the detection algorithm were calculated. In addition, the errors in the values of the hemodynamic characteristics calculated in the present studies were assessed.

Materials and Methods

Blood Pressure Registrations

The algorithm was evaluated in 24-hour blood pressure registrations obtained from a database of recordings in subjects that had been referred to the department of Internal Medicine I of the Dijkzigt Hospital for the assessment of hypertension (see Chapter 9). The mean age of these subjects (22 men and 1 woman) was 40 years (range, 18 to 60

years). They did not use any medication for at least 3 weeks before the blood pressure recording. The average 24-hour values (mean±SD) in these subjects were 105±14 mmHg for mean arterial pressure, and 75±9 bpm for heart rate.

A typical registration contains approximately 100,000 heart beats. Since editing of such a large number is not feasible, samples were taken from the registration by selecting 24 intervals of 36 seconds of blood pressure signal distributed equidistantly over the 24 hours of each registration. These intervals were selected by a computer program in such a way that they did not contain parts of the registration which were used earlier to optimize the filter for the detection of the dicrotic notch. In this way 864 seconds of signal, corresponding to approximately 1000 beats, were obtained from each subject.

Generation of Reference Beats

A dedicated editor EDIT was developed for the generation of reference values under visual inspection of the blood pressure signal. EDIT displays an overview of a 36 s signal fragment and two more detailed views of 8 s and 4 s, respectively (Figure 9). In both detailed views, the beats detected by ANALYSIS are marked by an indicator at the maximum pressure (*beat mark*). The start of the beat, the start position of the dicrotic notch and the end of the beat are indicated by *time marks*. In the most detailed view, the investigator can add or delete individual beats by adding or deleting beat marks. In addition, the position of the time marks can be changed. The 36 s view (upper panel) and

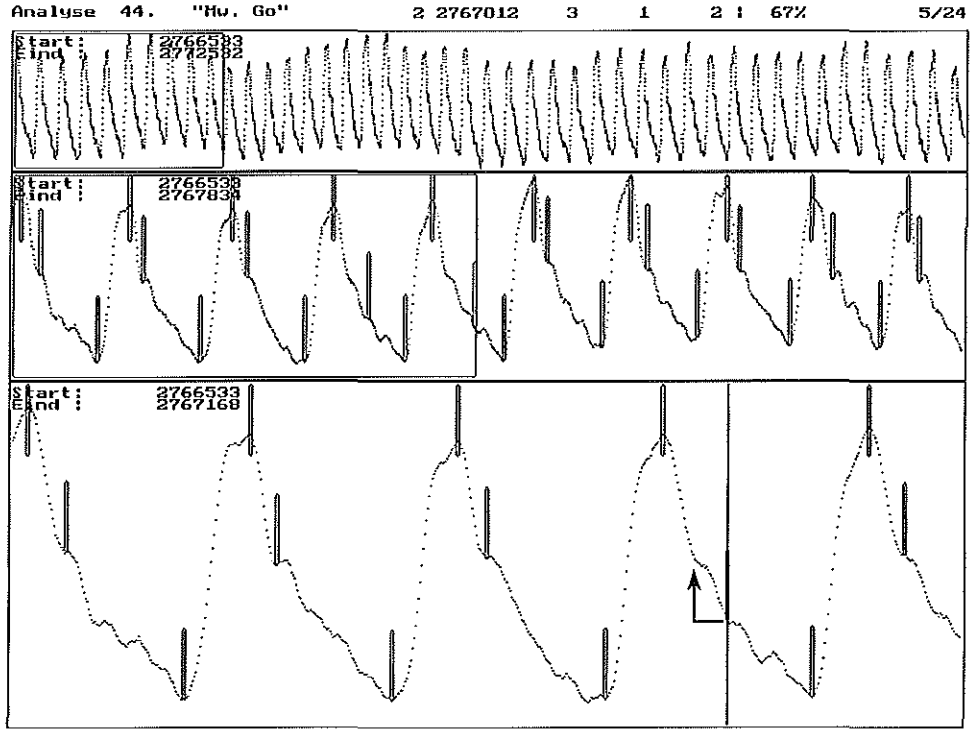

Figure 9. Screen display of the program EDIT that is used for the generation of reference beats. The position of the start of the dicrotic notch of the third complete beat in the lower panel as detected by ANALYSIS is to be corrected (arrow). The marker that indicates the position that is to be corrected has been selected by superposition of a hair line. The selected marker can be moved through the signal to the correct position. The name of the subject is fictitious.

the 8 s view (middle panel) serve as a display of the context of the beat that is edited in the 4 s view (lower panel).
After the editing process, the program parses the marks, checks for validity of user input, and calculates the hemodynamic parameters of the edited beats. These investigator-validated parameters (*reference beats* or *reference results*) are written to a file for later comparison with results obtained by ANALYSIS. Since both the position of the first and the last sample of a beat may be changed by the referee during the editing process, these positions cannot be used to find matching pairs of beats during the comparison of ANALYSIS results with reference beats. Instead, the position of the first maximum after the beginning of the beat was used as beat identification.

Sensitivity and Specificity

Results of a dichotomous classification method compared to a reference classification are commonly expressed in terms of sensitivity and specificity. Given N paired results

Table 1. Classification Parameters Used for the Calculation of Sensitivity and Specificity of the Detection Algorithm

		Method A +	Method A -	
Reference method R	+	TP	FN	TP+FN
	-	FP	TN	FP+FN
		TP+FP	TN+FN	N

TP true positive, TN true negative, FN false negative, FP false positive.

obtained by a dichotomous classification method A and a reference method R, a cross table of results can be constructed (Table 1), and sensitivity and specificity can be calculated according to (9).

$$Sensitivity_A = \frac{TP}{TP + FN}$$

$$Specificity_A = \frac{TN}{TN + FP} \qquad (9)$$

The sensitivity of the detection algorithm of ANALYSIS can be calculated according to this method, using the number of beats identified both by ANALYSIS and the referee as *true positive*, and the number of beats identified by the referee but not by ANALYSIS as *false negative*. However, this method does not allow the calculation of the specificity of the blood pressure analysis algorithm. Although the number of beats identified by ANALYSIS and rejected by the referee corresponds to *false positive*, the number of beats not identified by ANALYSIS nor by the referee, *true negative*, is unknown. To

circumvent this problem, the specificity was estimated by a time ratio (*time ratio specificity*), using the lengths of signal classified rather than the number of beats. The length of the signal containing no beats according to both ANALYSIS and visual inspection was used as true negative. The length of the signal containing beats according to ANALYSIS but not according to visual inspection was used as false positive. This length was calculated by adding the durations of the beats identified by ANALYSIS that were rejected during visual inspection. In order to assess the validity of this approximation, sensitivity was also calculated in this manner (*time ratio sensitivity*). The results of both methods were almost identical (Table 2), suggesting a reliable estimation of the specificity by the time ratio as well.

Error in the Estimation of Hemodynamic Parameters

The hemodynamic characteristics of the subjects under study were assessed by quantifying the diurnal profile and the short term variability of systolic pressure, mean arterial pressure, diastolic pressure, interbeat interval, heart rate, cardiac output, stroke volume and total peripheral resistance. The estimation of these characteristics by ANALYSIS was assessed by a comparison with characteristics calculated from reference beats. The comparison was not based on paired beat-to-beat differences, since such a comparison could only include beats that were detected both by the referee and the program which would bias the results.

For practical reasons, this evaluation could not be performed in the entire 24-hour blood pressure registration. Instead, the 24 periods of 36 s of blood pressure signal that were used for the calculation of sensitivity and specificity were used. This procedure generated a vast amount of statistics. Therefore, and since some results were very similar to each other, a selection from the results is presented. Blood pressure is represented by systolic pressure, being the most variable parameter, and by mean arterial pressure. The latter parameter is presented since it can be interpreted in correlation with cardiac output and total peripheral resistance. The results for diastolic pressure and enddiastolic pressure are very similar to the results of mean arterial pressure and are therefore omitted. The interbeat interval is presented rather than heart rate, since the interbeat interval can be derived directly from the signal and is intuitively more meaningful than the instantaneous heart rate. Results of cardiac output, stroke volume and total peripheral resistance are all presented. The following characteristics of each of these parameters are presented for the individual registrations.

» *24-hour average*

For systolic pressure, mean arterial pressure and interbeat interval, averages over each of the 24 periods of 36 s were calculated from reference values. The 36 s error was calculated as the difference between the ANALYSIS 36 s average and the reference 36 s average. Results are presented as mean±SD of the 24 reference 36 s averages and as the mean of the 24 corresponding 36 s errors. These values were not calculated for stroke volume, cardiac output and total peripheral resistance since the 24-hour values of both the reference method and ANALYSIS were by definition 100% (see Chapter 2).

» *nocturnal change*

In accordance to the studies in Chapter 9[9] and Chapter 10[10], the day was defined as the period between 8:00 hour and 20:00 hour, and the night was defined as the period between 0:00 hour and 6:00 hour. This definition was chosen to exclude the transience periods between the day and night. The nocturnal change was defined as the night-day

difference of the mean level over both periods and was calculated for the reference 36 s averages and for the 36 s average errors. The pooled standard deviation of the night-day difference was calculated according to

$$s_{night-day} = \sqrt{\frac{(n_{night}-1)s_{night}^2 + (n_{day}-1)s_{day}^2}{(n_{night} + n_{day} - 2)}},$$

with n_{day} and n_{night} the number of 36 s periods during the day and night, respectively, and s_{day} and s_{night} the standard deviation of the 36 s averages over the day and night, respectively. Results are presented as the night-day difference of the reference value, the pooled standard deviation of the night-day reference value and the night-day difference of the error.

» *24-hour average of short term variability*

For each registration, the standard deviation within each of the 24 periods of 36 s was calculated as a measure of short term variability. For each period the error was calculated as the ANALYSIS/reference ratio of 36 s standard deviations. Results are presented as mean±SD of the reference 36 s standard deviations and as the mean of the 36 s error. The results for stroke volume, cardiac output and total peripheral resistance are not calculated since they were not used in the present studies.

» *nocturnal change of short term variability*

Results are presented as the night-day difference of the reference 36 s standard deviations, the pooled standard deviation of the night-day difference, and as the night-day difference of the 36 s error. These values were not calculated for stroke volume, cardiac output and total peripheral resistance since the nocturnal changes in these parameters were not used in the present studies.

The individual results were summarized by group mean ± SD. Normal distribution was tested by the Shapiro Wilk statistic[11]. Significance of difference from zero was established at a 0.05 significance level, using a t-tests for normally distributed parameters and by a signed rank test[12] otherwise.

In addition, the correlation of the nocturnal change of mean arterial pressure with the nocturnal change of cardiac output and total peripheral resistance was quantified with the Pearson correlation coefficient in both the reference results and the ANALYSIS results. All statistical computations were perfomed with SAS statistical software (SAS V6.04 for Personal Computers, SAS Institute Inc., Cary, North Carolina, USA).

Results

Sensitivity and Specificity

Table 2 displays the lengths of registration parts as classified by visual inspection of the ANALYSIS results, and the derived values of time ratio sensitivity and time ratio specificity. In addition, the values of sensitivity based on the number of classified beats are shown. On the average, 817 s of the signal consisted of valid blood pressure signal (true positive plus false negative, individual data not shown), i.e., 94.6±5% of the registration (range, 75% to 98%; median 96%). The mean±SD of the time ratio sensitivity was 97.3±5.8% (range, 74.1% to 100.0%; median 99.3%). The values of sensitivity and time ratio sensitivity were almost identical. The time ratio specificity was 88.2±15.6% (range, 51.2% to 100.0%; median 93.5%). In eight registrations, the time ratio specificity was smaller than 90%. In all registrations, however, the number of erroneously detected

Table 2. Classification of ANALYSIS Results by Visual Inspection

Registration	True Positive (s)	False Negative (s)	True Negative (s)	False Positive (s)	Time Ratio Sensitivity (%)	Sensitivity (%)	Time Ratio Specificity (%)
1	823	7.2	19	14.7	99.1	99.3	56.5
2	815	17.2	27.8	4.1	97.9	96.9	87.2
3	831	12.8	20.1	0	98.5	98.5	100.0
4	823	1.1	20.4	19.5	99.9	99.8	51.2
5	812	11	21.7	19.2	98.7	98.7	53.1
6	784	2	55.3	22.5	99.7	99.7	71.1
7	790	5.2	53.7	14.9	99.3	99.4	78.3
8	693	101.9	64.5	4.3	87.2	85.9	93.7
9	612	33.4	189.1	29.8	94.8	95.2	86.4
10	811	35.6	17.5	0	95.8	97.0	100.0
11	841	0.6	19.9	2.2	99.9	99.9	89.9
12	841	1.2	21.7	0	99.9	99.8	100.0
13	845	0	18.7	0	100.0	100.0	100.0
14	843	0.6	20	0	99.9	99.9	100.0
15	828	7.7	25.5	2.7	99.1	98.8	90.4
16	838	2.5	21.7	1.5	99.7	99.7	93.5
17	563	197.3	98.7	4.9	74.0	78.2	95.3
18	842	0.7	21.2	0	99.9	99.9	100.0
19	806	33.3	23	1.7	96.0	97.2	93.2
20	821	8.3	32.6	2.4	99.0	98.8	93.2
21	842	0.6	21	0	99.9	99.9	100.0
22	806	4.7	53.6	0	99.4	99.5	100.0
23	795	1.5	65.4	2.4	99.8	99.7	96.5
Range	563-845	0-197.3	17.5-189.1	0-29.8	74.1-100.0	78.2-100.0	51.2-100.0
Mean±SD	796±74	21±44	41±39	6.4±8.9	97.3±5.8	97.2±5.2	88.2±15.6
Median	821	5.2	21.7	2.4	99.3	99.4	93.5

Table shows lengths of signal classified as true positive, false negative, true negative and false positive in the evaluation of ANALYSIS, and sensitivity and specificity calculated from time ratios. Sensitivity calculated from the number of beats classified is shown as well.

Table 3. Reference Values and ANALYSIS Errors for Systolic Pressure

Registration	Pressure Level (mmHg)				Short Term Variability (mmHg)			
	24-hour Average		Nocturnal Change		24-hour Average		Nocturnal Change	
	Reference	Error	Reference	Error	Reference	Error	Reference	Error
1	119±16	0.0	-31±7	0.0	4.6±2.3	1.00	-2.1±2.1	0.00
2	115±14	0.0	-21±11	-0.1	5.0±1.8	1.00	-1.5±1.7	0.00
3	109±11	0.0	-16±7	-0.2	6.1±3.0	1.00	-2.1±3.0	0.00
4	145±21	0.0	-42±8	0.0	5.1±2.3	1.00	-2.0±2.0	-0.01
5	149±28	0.0	-62±8	0.0	7.3±4.0	1.00	-4.7±2.3	0.01
6	155±16	0.0	-30±9	-0.1	6.8±2.5	1.00	1.5±2.7	0.00
7	156±29	0.0	-47±21	-0.1	8.8±3.3	1.00	-2.8±3.1	0.00
8	153±21	-0.3	-32±18	-0.2	6.0±2.6	0.98	-1.7±2.9	0.02
9	173±16	0.0	-12±18	0.0	5.7±2.0	1.00	2.0±2.1	0.01
10	141±24	-0.1	-40±16	-0.3	6.1±2.3	0.99	-2.7±2.0	-0.03
11	147±19	0.0	-36±10	0.0	6.1±2.6	1.00	-1.6±2.6	0.00
12	148±14	0.0	-29±8	0.0	6.1±2.5	1.00	1.1±2.5	0.00
13	171±21	0.0	-37±15	0.0	6.0±2.3	1.00	-0.9±2.6	0.00
14	161±28	0.0	-56±15	0.0	7.1±1.9	1.00	-0.3±1.9	0.00
15	149±24	0.1	-43±12	-0.3	7.6±3.5	0.99	-1.2±2.8	0.00
16	138±16	0.0	-27±12	-0.1	6.1±2.7	1.00	-0.3±2.2	0.00
17	125±13	-0.3	-16±10	-0.8	4.5±2.1	1.00	-2.2±2.1	0.11
18	129±20	0.0	-27±12	0.0	6.8±4.1	1.00	-3.3±4.4	0.00
19	140±24	0.0	-43±14	0.1	5.3±2.2	1.02	-3.2±1.6	0.06
20	145±12	-0.1	-2±8	0.0	9.1±2.4	0.99	0.8±1.9	0.00
21	132±12	0.0	-18±9	0.0	6.4±4.1	1.00	-4.1±3.3	0.00
22	126±11	0.0	-17±10	-0.1	4.9±2.5	0.99	0.6±2.7	0.01
23	136±12	0.0	-21±8	0.0	6.5±3.9	1.00	0.1±2.8	0.00
mean±SD	141.8±16.5	-0.03±0.09	-30.6±14.6*	-0.09±0.18†	6.27±1.17	1.00±0.01	-1.33±1.79‡	0.01±0.03§

*, $p<0.0001$; †, $p<0.001$; ‡, $p<0.01$; §, $p<0.05$

beats was smaller than 5% of the total number of detected beats (individual data not shown).

Error in the Estimation of Hemodynamic Parameters

The results of the evaluation of the quantitative characteristic estimation by ANALYSIS are listed in Table 3 to Table 6. The average error of the estimation of 24-hour average level of systolic pressure and mean arterial pressure was smaller than 0.1 mmHg (n.s.) with a largest error of -0.3 mmHg. There was a significant ($p<0.01$) but very small (<-0.1 mmHg) underestimation of average nocturnal change of blood pressure. In

Table 4. Reference Values and ANALYSIS Errors for Mean Arterial Pressure

Registration	Pressure Level (mmHg)				Short Term Variability (mmHg)			
	24-hour Average		Nocturnal Change		24-hour Average		Nocturnal Change	
	Reference	Error	Reference	Error	Reference	Error	Reference	Error
1	84±10	0.0	-17±4	0.1	3.6±2.2	1.00	-1.8±2.1	0.01
2	81±10	0.0	-15±7	0.0	4.0±1.6	1.00	-1.0±1.6	0.00
3	77±6	0.0	-6±6	-0.1	4.4±2.4	1.00	-0.9±2.7	0.01
4	115±17	0.0	-33±6	0.0	4.0±1.9	0.99	-2.0±1.7	-0.01
5	118±21	0.0	-46±6	0.0	5.1±2.9	1.00	-3.3±1.6	0.01
6	119±13	0.0	-25±7	-0.1	5.5±2.5	1.00	1.0±2.8	0.00
7	116±17	0.0	-27±12	-0.1	6.7±2.5	1.00	-2.1±2.4	0.00
8	121±18	-0.3	-27±15	-0.4	4.5±2.5	0.97	-1.5±2.8	0.02
9	129±12	0.0	-5±14	0.1	4.5±1.8	0.99	1.5±1.9	0.00
10	105±15	0.0	-24±11	0.0	4.3±2.1	1.00	-2.2±1.9	0.02
11	111±14	0.0	-24±9	0.0	5.1±2.2	1.00	-0.9±2.3	0.00
12	113±13	0.0	-26±7	0.0	4.6±2.4	1.00	-0.1±2.4	0.00
13	116±12	0.0	-21±8	0.0	4.0±1.8	1.00	-0.7±2.1	0.00
14	109±18	0.0	-34±9	0.0	4.5±1.6	1.00	0.0±1.6	0.00
15	114±17	0.0	-30±8	-0.2	6.3±2.7	0.99	-1.1±2.2	0.01
16	103±10	0.0	-15±9	-0.1	5.0±2.2	0.99	0.2±2.0	0.00
17	87±8	0.0	-7±7	0.0	3.1±1.5	0.96	-1.6±1.4	0.02
18	97±11	0.0	-18±8	0.0	5.1±2.8	1.00	-2.0±3.1	0.00
19	96±14	0.0	-20±9	0.1	4.0±1.8	1.01	-2.5±1.4	0.05
20	102±10	-0.1	-1±8	0.0	5.3±2.5	0.99	0.9±1.3	0.00
21	105±9	0.0	-12±7	0.0	5.1±3.1	1.00	-2.7±2.6	0.00
22	98±10	0.0	-13±10	-0.1	3.8±2.1	1.00	1.4±2.2	0.00
23	105±10	0.0	-17±7	0.0	5.5±2.8	1.00	0.8±1.8	0.00
mean±SD	105.2±13.6	-0.02±0.08	-20.1±10.5*	-0.04±0.10	4.7±0.85	1.00±0.01	-0.90±1.38†	0.01±0.01‡

*, $p<0.0001$; †, $p<0.001$; ‡, $p<0.05$

individual registrations the error was always less than 1.0 mmHg for systolic pressure and less than 0.5 mmHg for mean arterial pressure. The difference between the reference and ANALYSIS results for short term variability over the 24 hours was small (<1%) and non-significant. The maximal error was 2% for systolic pressure and 4% for mean arterial pressure. The nocturnal change of short term variability was, on the average, slightly (1%) overestimated ($p=0.01$). For 2 registrations, however, the overestimation was larger than 5% (for systolic pressure, 6% and 11%).

The errors in the values of the 24-hour average level and of the nocturnal change of

Table 5. Reference Values and ANALYSIS Errors for Interbeat Interval

Registration	Beat Length (ms)				Short Term Variability (ms)			
	24-hour Average		Nocturnal Change		24-hour Average		Nocturnal Change	
	Reference	Error	Reference	Error	Reference	Error	Reference	Error
1	661±140	-1	288±40	-3	37±27	1.00	36±26	-0.02
2	811±157	0	277±88	0	58±26	1.00	16±28	-0.01
3	895±217	-2	407±138	-8	73±41	0.99	31±45	-0.03
4	835±147	-1	263±60	-2	51±18	1.00	13±17	-0.01
5	720±92	0	98±69	0	47±18	1.00	-14±16	0.00
6	915±117	0	50±109	0	42±17	1.00	-4±20	0.00
7	644±87	0	160±54	1	29±10	0.98	11±7	-0.01
8	804±100	4	90±106	7	47±22	1.01	10±19	0.03
9	996±95	0	137±65	-2	45±20	0.98	-1±22	-0.02
10	707±137	-3	249±96	-11	62±41	0.98	44±40	-0.09
11	776±143	0	272±65	0	62±19	1.00	-3±21	0.00
12	846±115	0	146±104	0	53±25	1.00	18±24	-0.01
13	815±104	0	109±98	0	33±11	1.00	-1±11	0.00
14	729±121	0	227±79	0	30±19	1.00	38±11	0.00
15	738±134	-1	188±92	-2	60±27	1.00	28±28	-0.02
16	872±134	0	249±82	1	66±30	1.00	50±23	0.00
17	1241±178	-18	320±98	-44	54±25	0.88	-3±28	-0.27
18	783±75	0	75±58	0	28±9	1.01	-6±9	0.05
19	946±179	-2	360±88	-8	60±21	0.97	-3±21	-0.04
20	838±127	1	20±133	0	37±14	1.00	-3±9	0.00
21	813±144	0	213±103	0	47±19	1.00	1±16	0.01
22	984±134	-1	125±128	0	79±29	0.98	16±23	-0.01
23	733±112	0	121±75	0	45±19	1.00	17±20	0.00
mean±SD	831±130	-1.0±4.0	193±103*	-3.1±9.7	49.8±13.9	0.99±0.03§	12.7±17.9†	-0.02±0.06‡

* $p<0.0001$, † $p<0.01$, ‡ $p<0.05$ for difference with zero; § $p<0.05$ for difference with 1.0

interbeat interval were small (-1 ms and -3 ms, respectively) and insignificant. There was a slight underestimation of short term variability (1%, $p<0.05$) and of nocturnal change of short term variability of this parameter (-2%, $p<0.05$). For registration 17 however, there was a considerable underestimation of 24-hour average level, nocturnal change, short term variability and nocturnal change of short term variability of the interbeat interval.

The average and median errors of the nocturnal change for cardiac ouput, stroke volume and total peripheral resistance were 1.5% (median error 0.8%; reference -26.3%, $p<0.05$), 1.7% (median error 0.8%, reference -5.6%, $p<0.05$), and -2.4% (median error, -0.8%;

reference 8.7%, $p<0.001$), respectively. The median errors appeared to be small compared to the reference values. There was, however, a considerable scatter of the error in individual registrations. These appeared to be significant (two-tailed t-test, significance at $p<0.05$; data not shown) for registrations 3 and 9 for cardiac output, registrations 5 and 9 for stroke volume, and registrations 1, 3 and 9 for total peripheral resistance. In these instances, the nocturnal changes of cardiac output and stroke volume were overestimated and the nocturnal change of total peripheral resistance was underestimated. The group average errors of these parameters were strongly determined by these registrations.
The Pearson coefficient for correlation within the group of the nocturnal change of mean arterial pressure with the nocturnal change of cardiac output was 0.42 ($p=0.046$) for reference values and 0.49 ($p=0.02$) for ANALYSIS results. The correlation coefficients of the nocturnal change of mean arterial pressure with the nocturnal change in total peripheral resistance were 0.16 (n.s.) for reference values and 0.12 (n.s.) for ANALYSIS results.

Table 6. Reference Values and ANALYSIS Errors for Nocturnal Change in Cardiac Output, Stroke Volume and Total Peripheral Resistance

Registration	Nocturnal Change in Cardiac Output (% 24-Hour Average)		Nocturnal Change in Stroke Volume (% 24-Hour Average)		Nocturnal Change in Total Peripheral Resistance (% 24-Hour Average)	
	Reference	Error	Reference	Error	Reference	Error
1	-49±14	2.9	-13±18	4.6	49±26	-17.1
2	-25±19	-1.2	7±12	-0.8	4±15	0.5
3	-31±8	6.7	10±19	7.9	26±12	-7.4
4	-37±15	1.8	-11±10	2.1	10±10	-1.9
5	-40±10	3.3	-30±8	3.7	4±8	-3.5
6	-8±13	0.8	-4±8	0.9	-12±12	-0.8
7	-36±16	-1.4	-14±13	-1.4	15±10	0.7
8	-18±20	-4.6	-8±13	-3.8	-6±14	3.7
9	-20±14	13.3	-7±9	14.1	16±12	-12.6
10	-40±22	0.5	-10±15	-1.1	20±19	-1.3
11	-35±16	-2.7	-2±11	-2.8	11±15	2.8
12	-6±14	1.0	12±8	1.2	-19±11	-0.8
13	-23±24	-0.1	-8±14	-0.1	0±20	0.2
14	-48±20	3.1	-23±11	4.0	20±14	-4.1
15	-30±19	-0.5	-8±9	-0.1	3±13	-0.7
16	-35±14	3.2	-10±12	4.3	22±11	-3.4
17	-23±14	4.0	1±8	0.8	16±13	-4.4
18	-15±13	-0.5	-7±11	-0.5	-3±12	0.5
19	-45±19	3.4	-14±13	2.9	29±14	-3.5
20	-2±9	1.3	-2±12	1.7	1±8	-1.2
21	-25±15	0.2	-2±16	0.3	13±13	-0.2
22	-8±10	0.1	4±17	0.0	-6±12	-0.5
23	-8±15	0.1	9±9	0.1	-11±15	-0.2
mean±SD	-26.3±14.0*	1.5±3.5†	-5.6±10.3†	1.7±3.8†	8.7±15.6†	-2.4±4.7‡

* $p<0.0001$, † $p<0.05$, ‡ $p<0.001$ for difference with zero

Discussion

Sensitivity and Specificity

For the evaluation of the performance of ANALYSIS, signal fragments were selected from 23 blood pressure registrations. The signal fragments were obtained in 24 samples distributed equidistantly over each registration. In this way, a total signal length of five hours and 30 minutes was evaluated. The signal fragments appeared to be of good quality; more than 95% of the signal contained valid beats as judged by visual inspection. This result is not representative for a random blood pressure recording obtained by means of the Oxford Medilog system, since the registrations were obtained from our database of registrations, and recordings of poor signal quality are not included in the studies and are therefore not present in our registration database. The registrations used in this evaluation, however, were not selected on signal quality and may therefore be considered as representative of the database and of the studies performed in our centre.

Since it was not possible to calculate the specificity of the ANALYSIS program on the basis of the number of beats, sensitivity and specificity were calculated from the length of registation classified. The values of sensitivity that were calculated from the number of beats classified were almost identical to the values of sensitivity based on time ratios, suggesting a good estimation of specificity as well by time ratio specificity. The sensitivity of ANALYSIS was satisfactory with a median value of 99.3%. The median value of specificity was 93.5%. For 15 registrations, the specificity was larger than 90%, and in these registrations less than 1% of the total number of beats detected by ANALYSIS was detected erroneously. In eight registrations, the specificity was smaller than 90%. In these registrations, 10% to 49% of the signal fragments that were classified by visual inspection as being of insufficient qualtity or containing artifacts were classified as valid signal by ANALYSIS. Since the largest part of the registration was of good qualtity, the number of erroneously detected beats as compared to the total number of detected beats was small. In one of these eight registrations (registration 9), 5% of the total number of beats as detected by ANALYSIS consisted of erroneously detected beats, in the other 7 registrations this was less than 3%. The quantitative effect will be discussed below.

Error of the Estimation of Hemodynamic Parameters

The errors of 24-hour average of blood pressure (<0.3mmHg) and of the night-day blood pressure change (<0.8mmHg) in the individual registrations appeared to be small compared to the intrinsic variation of blood pressure. The short term variability of blood pressure was estimated with an accuracy better than 2%, and the nocturnal change of short term variability of blood pressure with an accuracy better than 5%, except in two registrations (6% and 11%). In the group, the error was very small and in most cases non-significant.

With the exception of registration 17, the errors in the estimation of the interbeat interval parameters were small. For registration 17 there was a considerable underestimation of 24-hour average level, nocturnal change, short term variability and nocturnal change of short term variability of interbeat interval. The heart rate of this subject was unexpectedly low during the whole registration, and it appeared that a 36 s period at the end of the night was rejected by the analysis program, since the beat length during that period was longer than the artifact rejection limit of ANALYSIS.

Although the median error of the nocturnal change of cardiac output, stroke volume and total peripheral resistance were small compared to the reference values, four registrations (numbers 1, 3, 5, and 9) caused a considerable increase of the group average error for these parameters. These registrations contained approximately 5-10% of beats in which the position of the dicrotic notch was detected too late (see Figure 12 for an example). These errors occur mainly during the night, causing an overestimation of stroke volume and cardiac output during the night, and therefore an underestimation of the absolute value of the decrease of these parameters during the night. This can be explained by the lower heart rate and therefore the lower values of the first and second derivative of the signal during the night. The number of these errors can probably be diminished by limiting the search to the part of the beat that contains the dicrotic notch, using nomograms for the duration of the systole, and applying a beat length dependent threshold value instead of zero passing.
In addition to these errors, registrations 5 and 9 contained fragments of damped signal in which not all abnormal waveforms were rejected by ANALYSIS.

References

1. Smith NT, Wesseling KH, Weber JAP, de Wit B: Preliminary evaluation of a pulse contour cardiac output method in man: feasibility of brachial or radial arterial pressures. Proc San Diego Biomed Symp. 1974;13:107-113
2. Wesseling KH, de Wit B, Weber JAP, Smith NT: A simple device for the continuous measurement of cardiac output. Adv Cardiovasc Phys 1983;5:16-52
3. Sprangers RLH: On the role of cardiopulmonary receptors at the onset of muscular exercise. Amsterdam: University of Amsterdam, 1990. Dissertation
4. Stok WJ, Baisch F, Hillebrecht A, Schulz H, Meyer M, Karemaker JM: Noninvasive cardiac output measurement by arterial pulse analysis compared with inert gas rebreathing. J Appl Physiol 1993;74:2687-2693
5. Wesseling KH, Jansen JRC, Settels JJ, Schreuder JJ: Computation of aortic flow from pressure in humans using a nonlinear, three-element model. J Appl Physiol 1993;74:2566-2573
6. Jansen JCR, Smith NT, Wesseling KH, Settels JJ, Schreuder JJ: Continuous cardiac output monitoring by pulse contour during cardiac surgery. Eur Heart J 1990; 11(suppl 1):26-32
7. Cornbleet PJ, Gochman N: Incorrect least-squares regression coefficients in method-comparison analysis. Clin Chem 1979;25:432-438
8. Van Bemmel HJ: Biological signal processing. In: Ingram D, Bloch RF, eds. Mathematical methods in medicine. New York, John Wiley&Sons, Ltd., 1984; 225-272
9. Idema RN, van den Meiracker AH, Balk AHMM, Bos E, Schalekamp MADH, Man in 't Veld AJ: Decreased circadian blood pressure variation up to three years after heart transplantation. Am J Cardiol 1994;73:1006-1009

10. Idema RN, van den Meiracker AH, Balk AHMM, Bos E, Schalekamp MADH, Man in 't Veld AJ: Abnormal diurnal variation of blood pressure, cardiac output, and vascular resistance in cardiac transplant recipients. Circulation 1994;90:2797-2803
11. Shapiro SS, Wilk MB: An analysis of variance test for normality (complete samples). Biometrika 1965;52:591-611.
12. Lehmann EL: Nonparametrics: Statistical methods based on ranks. San Francisco, Holden-Day, Inc., 1975

Chapter 4

A New Model for Diurnal Blood Pressure Profiling - The Square Wave Fit Compared With Conventional Methods

R.N. Idema, E.S. Gelsema, G.J. Wenting, J.L. Grashuis, A.H. van den Meiracker, M.L. Brouwer, and A.J. Man in 't Veld

Published in Hypertension 1992;19:595-605

Supported by a grant-in-aid for scientific research from the Netherlands Heart Foundation (NHS 86.070) and by the Cardiovascular Research Foundation Rotterdam.

Summary

For the characterization of diurnal blood pressure variation, we developed a simple mathematical model which nevertheless does justice to the specific form characteristics of individual blood pressure registrations. Analysis was based on 24-hour continuous intra-arterial measurements of blood pressure obtained in 23 hospitalized patients with mild to moderate untreated essential hypertension (mean±SD of mean arterial pressure 112±13 mmHg). The day-night difference for mean arterial pressure varied markedly (mean 18.6 mmHg, range 6.8 - 36.0). Inspection of the profiles suggested a model of blood pressure consisting of two contiguous, complementary periods of constant pressure, a so-called square wave. Determination of the times of transience between both periods (segmentation) was performed individually, using a least square error criterion. Results were compared to those obtained by conventional methods including analysis by Fourier modeling. The square wave fit accounted for a larger fraction (66%) of circadian variance of mean arterial pressure than modeling based on segmentation by visual inspection (59%, considerable observer bias) or by clock time (50%). Application of the Minnesota Cosinor method resulted in the poorest description (47%). Segmentation based on harmonic modeling (61%) appeared to be cumbersome (10 harmonics needed), and the significance of additional information offered over the square wave fit is dubious. Observer bias makes segmentation by visual inspection unsuitable for assessment of the circadian variance of blood pressure. Even when daily activities are strictly regulated (hospital environment), circadian variance is not well modeled by clock time. As compared to harmonic analysis, square wave fitting is simple, and it appears to best model the circadian variance. The method can also be applied to data obtained from non-invasive ambulatory blood pressure monitoring.

Introduction

Blood pressure tends to be highest in the morning, decreasing gradually during the day to reach its lowest values at night. The actual blood pressure pattern found is strongly influenced by factors like the amount of physical activity and periods of rest[1,2]. Nevertheless, a constant and prominent feature in healthy men is a fall of blood pressure during the night, which constitutes a major fraction of the total diurnal blood pressure variation[3]. The question as to whether there is an internal biological "blood pressure clock" analogous to the one responsible for the circadian variation of certain hormone levels and body temperature is still open. Various pathologies have been shown to alter circadian blood pressure fluctuations. For instance, a paradoxical rise in blood pressure at night was found in patients with sleep apnoea syndrome[4], preeclamptic toxemia[5] and autonomic neuropathy[6]. A more or less constant 24-hour blood pressure level was observed in patients with malignant hypertension[7], pheochromocytoma[8], and Cushing's syndrome[9]. Also after heart transplantation an abnormal circadian blood pressure profile has been described[10]. Since in these patients it appeared to be very difficult to unravel the

quantitative effect of various intrinsic and extrinsic factors on blood pressure level, there is a considerable interest in an objective quantitative description of the circadian blood pressure variation.

In most analyses, characterization of 24-hour blood pressure was based on averaging records at fixed clock times or, slightly better, after synchronizing records relative to the time of waking. These procedures segment the profile into a high-pressure period, roughly corresponding to the day, and a low-pressure period corresponding to the night. The difference in mean blood pressure value over the two periods then corresponds to circadian variation.

Obviously, proper and objective description of 24-hour blood pressure variation is served by a mathematical model. The Minnesota Cosinor method[11], which has been used most often, is probably one of the least accurate methods since the basic assumption that the high- and low- pressure periods have the same length is incorrect and violates the reality of the diurnal life cycle. Nowadays, most investigators agree that the reported sinusoidal pattern of blood pressure variation probably is an artifact caused by averaging individual records without synchronization for such variables as time of sleep, time of waking, or type of activity. An alternative approach is Fourier analysis, a technique by which the individual 24-hour blood pressure profile is modeled as a superposition of cosine functions. Recently, in a report describing blood pressure profiles of shift workers, Chau et al[12] reported such a method. By statistical analysis of their 24-hour blood pressure data, they found that four harmonics were needed to model the profile. However, when more harmonics have to be used, as will be shown to be the case in our material, the interpretation of the synthesized signal, and therefore the determination of blood pressure shifts, is rather cumbersome.

In the present study we describe a new mathematical model for the characterization of 24-hour blood pressure profiles. This model describes the profile as a square wave with exactly one period of high pressure and one period of low pressure. The duration of the two periods is only constrained by the requirement that their sum equals 24 hours. We applied this model to a collection of 23 recordings of 24-hour intra-arterial blood pressure measurement and compared the results with those obtained by other methods.

Methods

Subjects and Blood Pressure Measurements

In a group of 23 male subjects with moderate essential hypertension (mean ± SD of 24-hour mean arterial pressure, 112±13 mmHg; range, 86-138 mmHg), blood pressure was recorded continuously for a period of 24 hours. All patients gave informed consent to undergo intra-arterial measurements, which were approved previously by the Ethical Committee of the Dijkzigt Hospital. None of the subjects had received treatment for hypertension for at least 3 weeks. All subjects were otherwise healthy. During blood pressure registrations, patients were hospitalized, but were not restricted in their activities. Blood pressure registrations were obtained by means of the Oxford technique[13] by which blood pressure is continuously measured via a catheter in the brachial artery and is recorded on an Oxford Medilog II recorder (Oxford Medical Systems, Oxon, England). The catheter is permanently flushed with a heparin solution to prevent clotting. Recorder,

transducer, and perfusion unit are worn at heart level, which allows free movement of the patient.

Preprocessing of the Blood Pressure Signal

Blood pressure registrations were replayed and read into an Olivetti XP/7 computer system (Olivetti, Ivrea, Italy) with a sampling frequency of 160 Hz. For this procedure an AT-Codas AD-converter (Dataq Instruments, Akron, Ohio, U.S.A.) with an accuracy of 12 bits was used. Subsequently, the blood pressure signal was analyzed by a computer program, which calculated systolic pressure, diastolic pressure, mean arterial pressure, and pulse interval of each beat.

A 24-hour blood pressure registration consists of about 100,000 heart beats. Such a large number is rather impractical for analysis. Moreover, the data contain short-term fluctuations, e.g., the respiratory blood pressure variation. These fluctuations are evidently not to be considered as a part of the circadian variation, and their effect should be excluded when various models of circadian variation are compared quantitatively. For these reasons, we calculated 20-minute averages of blood pressure and heart rate values and used these values as input for further analysis. A time period of 20 minutes was chosen to attain a substantial data reduction while preserving the form characteristics of the circadian blood pressure variation, as was judged by visual inspection.

Blood Pressure Modeling

For analysis of the 24-hour blood pressure profiles, a new model was developed and applied. The method based on this model, i.e., fitting a square wave to each individual profile, is outlined in detail below. Results were compared with existing methods such as segmentation based on visual inspection of records by two independent observers, segmentation by clock time, the Cosinor Method, and Fourier modeling as reported by Chau et al[12]. Since these methods have been described in detail elsewhere, they are summarized only in an Appendix. The method introduced by Chau, however, will be discussed in detail, since as part of the present research, its applicability was carefully analyzed.

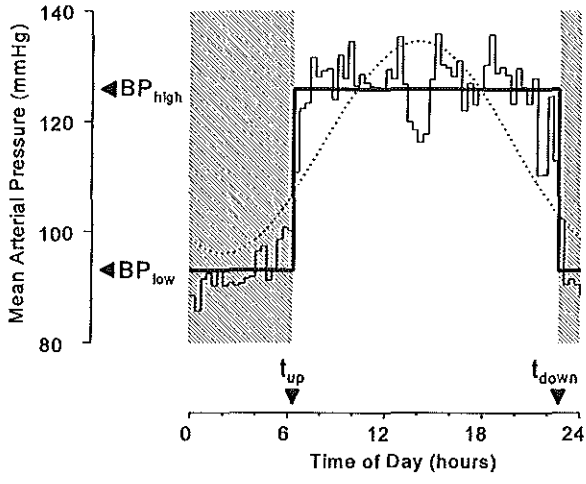

Figure 1. Representation of a 24-hour profile of mean arterial blood pressure (MAP) (thin line), being a typical example from our patient group. A square wave fit (thick line) and its corresponding parameter values are shown. Hatched area indicates "night" as indicated by the square wave fit. Dashed line represents a cosinor fit.

The square wave fitting method

Figure 1 shows a 24-hour profile of mean arterial pressure, being a typical example from the patient group. Visual inspection

of the individual profiles shows a marked fall of blood pressure during the evening and the night. Transition from the period of low to high blood pressure is rather abrupt. In contrast, the transition from high to low blood pressure appears to be more gradual and also the length of the period of low pressure differs from profile to profile, although it is generally shorter than the period of high pressure. These observations on individual profiles suggest a square wave model of 24-hour blood pressure. In this model, a 24-hour blood pressure profile is represented by a wave form consisting of two alternating contiguous periods of low and high pressure. There are no restrictions to the duration of these periods, except that their sum equals 24 hours.

To find the best fitting square wave for a given blood pressure profile, cross correlation values of the profiles with all possible different square waves are determined. The best fitting square wave is identified by the highest cross correlation value. The square waves are generated as discrete functions, only defined at the time points corresponding to actual measurements. Thus, for a blood pressure profile consisting of N equidistant samples (in this material 72 mean values over 20 minutes each), N*(N-1) different square waves exist. This can easily be verified by considering that the minimal length of the low pressure period is 1 time unit (20 minutes), the maximal length of the low pressure period is N-1 time units, and that for each of these alternatives, there are N different possibilities for the phase of the square wave.

As a first step, both the blood pressure profile and each square wave are standardized to a mean of zero and a standard deviation of 1.0. The purpose of this scaling operation is to standardize outcomes of the cross correlation values to a range from 1.0 to -1.0. A value of 1.0 corresponds to a perfect fit, whereas a value of -1.0 corresponds to the worst possible fit. Subsequently, for each square wave the cross correlation value is calculated as the average product of the 72 corresponding values of the square wave and the blood pressure profile. The maximum among the N*(N-1) values obtained in this way, cc_{max}, identifies the best fitting square wave. This square wave is used to segment the original blood pressure profile in a high and a low pressure period. The fit resulting from this procedure is optimal with respect to the square error. The squared value of cc_{max} expresses the fraction of total variation of the 24-hour blood pressure profile accounted for by the model. The percentage of total 24-hour variability (PVA) accounted for by the fitted square wave may therefore be expressed as

$$PVA = cc_{max}^{2} * 100$$

The square wave model is characterized by a set of four parameters: t_{up}, time point of passage of blood pressure from the low to the high pressure period; t_{down}, time point of passage of blood pressure from the high to the low pressure period; BP_{high}, observed mean blood pressure during the high pressure period; BP_{low}, observed mean blood pressure during the low pressure period. These parameters are illustrated in Figure 1. Other parameters may be calculated from these: BP_{mean}, 24-hour mean pressure; t_{up} -t_{down} , low blood pressure period; BP_{high}-BP_{low}, difference of mean observed blood pressure during the high and low pressure period. The circadian variation around BP_{mean} is characterized by three parameters: BP_{high} - BP_{low}, t_{up}, and t_{down}.

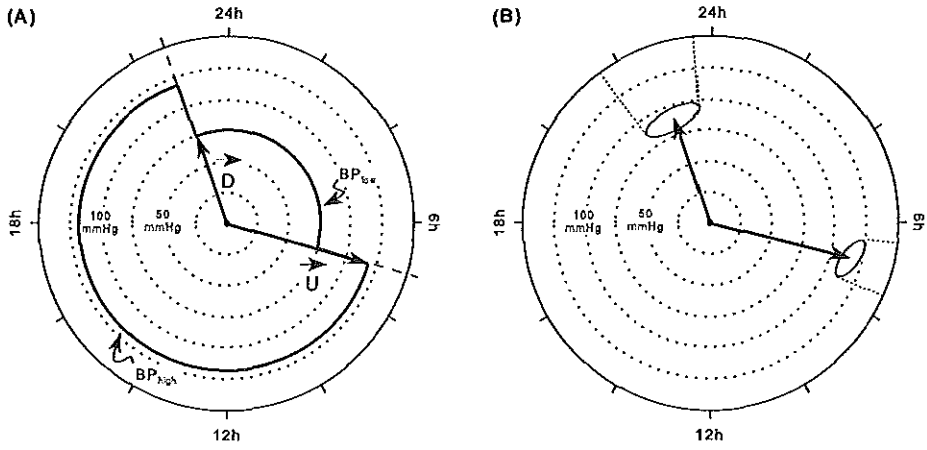

Figure 2. Representation of square wave parameters of a 24-hour blood pressure profile with a modeled high and low pressure span of 115 mmHg and 75 mmHg respectively, and a time of passage to the high and low pressure period of 7:00 hour and 23:00 hour, respectively. The two vectors (U and D) define a circular representation of the modeled blood pressure profile (virtual data, panel A). Mean vectors from our patient group and corresponding 95% confidence regions are shown in Panel B.

Since time of day is periodic, a circular representation, comparable to the cosinor display technique[11] can be used. In such a representation, a square wave is characterized by two vectors, $\underline{U}$ and $\underline{D}$, originating from the midpoint of a circle. The angles of the vectors with the vertical direction correspond to t_{up} and t_{down} respectively, whereas their lengths correspond to BP_{high} and BP_{low}. These two vectors define a circular representation of a square wave, as is demonstrated in Figure 2A. For a sample of square waves, a confidence ellipse for the mean vectors $\underline{U}_{mean}$ and $\underline{D}_{mean}$ may be calculated assuming a bivariate normal distribution.[11,14] For an illustration based on our patient material, see Figure 2B. Significance of the mean vectors is established if the confidence ellipses do not overlap the midpoint of the circle. For the classification of individual square wave parameters, a reference ellipse may be calculated. Blood pressure profiles with square wave parameters outside the reference area are to be considered as not belonging to the same class[15].

The square wave model assumes a bimodal distribution of 24-hour blood pressure. To establish whether such a distribution could be demonstrated in our subjects, a group histogram of 24-hour blood pressure was constructed after standardization of the individual profiles to the same mean and standard deviation. For these two parameters, the group averages of mean and standard deviation in individual profiles were substituted.

Most studies on circadian blood pressure variation are based on cuff measurements. The use of incidental measurements, however, has a detrimental effect on the precision, and in some cases, on the accuracy of model parameter estimation. To assess the sensitivity of the square wave fit to these effects, 200 profiles consisting of incidental measurements were simulated for each subject as follows. Of each blood pressure profile, mean and standard deviation of 20-minute periods were known. Single measurements for each period were generated by drawing at random from the normal distributions defined by

these parameters, a so-called Monte Carlo experiment. Square wave parameters obtained from these 200 profiles were compared with values obtained from the corresponding profile consisting of continuous measurements. Mean and standard deviation of these differences were calculated in each individual, and subsequently averaged over the group. These parameters indicate the inaccuracy and imprecision, respectively, which are introduced by the use of incidental measurements for the average subject. Also, the standard deviation of the mean difference over the group was determined over the 200 experiments. This parameter indicates the imprecision in the group mean.

Comparison to Conventional Methods

Obviously, segmentation of 24-hour blood pressure in two periods is a simplification of reality, and therefore a patent "truth", on the basis of which the methods can be verified, does not exist. However, the plausibility of the times of transience between the high and low pressure period, as obtained by square wave fitting and by harmonic analysis, can be assessed by a comparison to the results of visual inspection. We assumed that if two observers agreed about a value for t_{up} or t_{down}, this was a plausible time of transience. However, since we used 20-minute averages of blood pressure, a difference of 20 minutes between results should be allowed, and in such a case both values were considered to be plausible transience times. In all other cases, it was assumed that there was no single evident value for the time of transience. In these cases, plausibility of t_{up} or t_{down} could not be verified, and the recordings were excluded from the comparison.

We used the remaining values to determine whether the results of the various models were biased. For this purpose, we compared model values of t_{up} and t_{down} to average observer results. Significance of a tendency toward "later" or "earlier" values was tested two-sided at a probability level of 0.05 (sign test with ties[16]). The bias was assessed quantitatively by calculating the mean and standard deviation of the differences.

Another method to compare the appropriateness of the various models is based on comparing the percentage of total variance in 24-hour blood pressure profiles that is characterized by each model (PVA); for the square wave fit, it was calculated directly from cc_{max}. For the other models it was calculated according to its definition

$$PVA = 100 * \frac{Variance_{model}}{Variance_{actual}}$$

A model that uses more characteristics can be expected to explain more of circadian variation than a model that uses fewer parameters. When models are compared in this way, the number of parameters that a model uses has therefore to be taken into account.

Results

Visual Inspection

The time points of transience from the low to the high pressure period as determined by both observers show a good correspondence (Figure 3). In three cases, observer 2 indicated these points of transience earlier than observer 1, and in none of the cases later

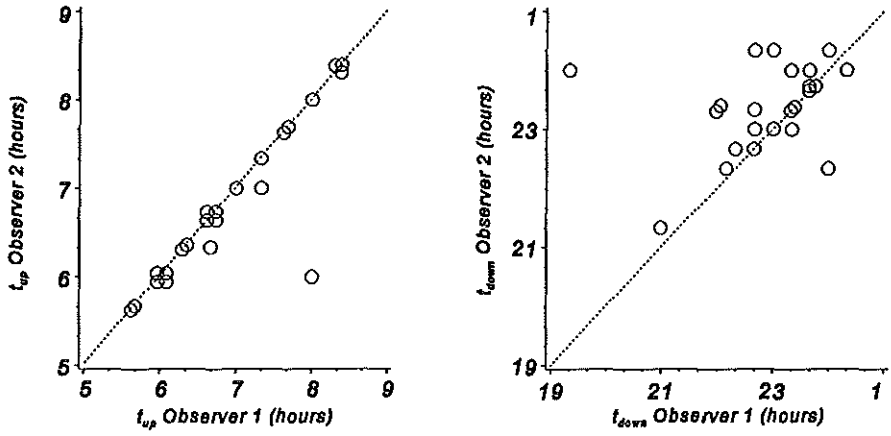

Figure 3. Scatterplots show comparison of t_{up} (left panel) and t_{down} (right panel) as indicated by two independent observers. Dashed line is the line of identity. Overlapping values are slightly shifted. t_{up} and t_{down}, time of transience to the high and low blood pressure period, respectively.

(not significant). In only one case was this difference more than one 20-minute period. On the average, the difference between observers is very small, having a mean value±SD of 0:07±0:25 hours. For t_{down}, however, this correspondence is worse. In 12 cases, observer 2 indicated a later value for t_{down} than observer 1, and in three cases an earlier value. This indicates a bias of observer 2 toward later values for t_{down} ($p<0.05$). For eight values, this difference is more than 20 minutes. For all 23 subjects, the mean value ±SD of the difference is 0:29±1:09 hours.

Only the cases in which observers differed no more than 20 minutes about the transience times were used to test the plausibility of square wave and Fourier modeling outcomes. This reduces the number of values available to 22 for t_{up} and to 15 for t_{down}.

Square Wave Method

Table 1 displays results of square wave fitting of the blood pressure profile of the 23 subjects together with their mean and standard deviation. Of the values of t_{up}, 90% are in the range 5:30 to 8:30 hour. The distribution of t_{up} in this range seems uniform. Of the values of t_{down}, 90% are in the range between 20:00 and 0:30 hour. The distribution of t_{down} is skewed: most blood pressure profiles have a time of passage from the high to the low pressure span between 23:00 and 0:00 hour, but in some profiles this passage occurs 2-3 hours earlier; in none does this occur more than half an hour later. The distribution of cc_{max} and PVA is also skewed. Low values of PVA and cc_{max} seem to coincide with low values of BP_{high}-BP_{low}, but have no evident relation with t_{up} or t_{down}. Figure 2B is a graph representative of mean square wave parameters of our group.

We compared the values of t_{up} and t_{down} obtained by square wave fitting to corresponding values obtained by visual inspection (Figure 4A, Table 2). In 21 of 22 profiles, values of t_{up} were similar. For t_{down}, this was the case in 10 of 15 profiles, with a bias toward later values than observers. For both parameters, the mean difference with observer results was

Table 1. Results of Square Wave Fitting to Individual Profiles of Mean Arterial Blood Pressure

Subject	t_{up} (h:m)	t_{down} (h:m)	t_{up}-t_{down} (h:m)	BP_{high} (mmHg)	BP_{low} (mmHg)	BP_{high}-BP_{low} (mmHg)	BP_{mean} (mmHg)	cc_{max}	PVA (%)
1	8:00	23:20	8:40	109.2	93.1	16.14	103.4	0.75	56.4
2	7:00	0:20	6:40	107.2	81.5	25.61	100.0	0.87	75.5
3	8:20	22:20	10:00	94.1	83.2	10.87	89.6	0.80	64.7
4	8:20	23:20	9:00	110.0	92.7	17.28	103.5	0.83	68.6
5	6:20	23:20	7:00	143.5	124.3	19.21	137.9	0.79	62.9
6	7:40	21:20	10:20	139.5	127.5	12.00	134.3	0.72	51.7
7	6:20	22:40	7:40	125.9	93.1	32.77	115.4	0.94	88.4
8	6:40	19:40	11:00	129.1	105.9	23.22	118.5	0.86	74.5
9	6:00	23:40	6:20	131.7	99.3	32.42	123.2	0.79	62.9
10	5:40	20:00	9:40	116.6	91.3	25.31	106.4	0.89	78.5
11	5:40	0:20	5:20	131.7	121.5	10.25	129.5	0.46	21.3
12	6:40	0:20	6:20	119.3	95.8	23.52	113.1	0.90	80.6
13	6:40	23:20	7:20	106.2	88.8	17.41	100.9	0.79	62.7
14	6:00	20:00	10:00	124.4	105.0	19.44	116.3	0.80	64.6
15	6:00	20:00	10:00	119.2	91.4	27.80	107.6	0.88	76.6
16	7:20	0:00	7:20	107.9	91.2	16.73	102.8	0.73	52.9
17	9:40	21:40	12:00	104.5	93.1	11.44	98.8	0.60	36.1
18	8:40	19:33	13:20	131.8	109.9	21.98	119.6	0.87	76.0
19	6:40	0:20	6:20	117.5	90.5	27.07	110.4	0.85	71.9
20	7:00	23:40	7:20	130.3	92.2	38.11	118.7	0.94	88.2
21	8:20	0:20	8:00	89.5	78.6	10.96	85.9	0.69	48.3
22	7:40	23:40	8:00	130.6	100.6	30.05	120.6	0.88	77.1
23	6:20	23:00	7:20	121.9	91.2	30.71	112.5	0.89	79.9
Mean	7:05	22:37	8:29	119.2	97.5	21.75	111.7	0.81	66.1
SD	1:04	1:43	2:01	13.9	13.0	8.05	13.1	0.11	16.2

BP_{mean}, average 24-hour mean arterial pressure; BP_{high}, Bp_{low}, average mean arterial pressure observed during the high and low pressure period, respectively; t_{up}, t_{down}, times of transience to the high and low pressure period, respectively; cc_{max}, cross correlation value of best fitting square wave; PVA, percentage of 24-hour variance modeled.

negligibly small, but for t_{down} there was a considerable scatter.The standardized group histogram of 24-hour blood pressure (Figure 5) clearly demonstrates a bimodal distribution of blood pressure in our patient group. About one third of the samples is contained in a small peak, which represents the distribution of pressures up to 104 mmHg. This peak has a median value of approximately 96 mmHg. About two thirds of the samples is contained in a larger peak, which has a median value of approximately 118 mmHg. The median values in the distributions are similar to the average values of BP_{low} and BP_{high} as determined by square wave analysis, which are 97.4 and 119.2 mmHg, respectively.

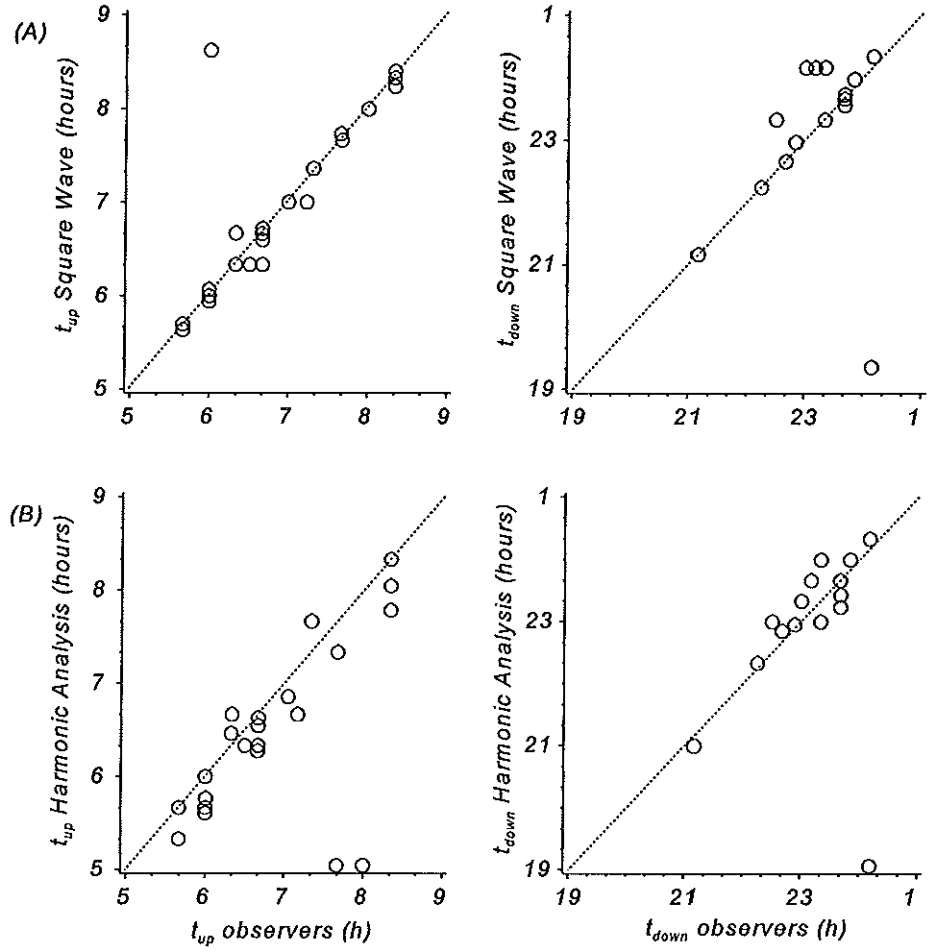

Figure 4. Scatter plots show comparison of transience times as indicated by square wave modeling (Panel A) and by harmonic analysis (Panel B) to average results of visual inspection. Only times on which both observers agree within 20 minutes are included in the plot. In Panel A, overlapping values are slightly shifted. t_{up} and t_{down}, time of transience to the high and low blood pressure period, respectively.

The sensitivity of square wave parameters to the use of incidental measurements is demonstrated in Table 3. The estimation of square wave parameters from incidental measurements is unbiased; the scatter in the estimation of individual and group parameters, however, is considerable.

Segmentation Based on Modeling With Multiple Harmonics

We determined the number of harmonics to be used in the Fourier model according to the two criteria that are described in the Appendix. In Table 4, runs-test values for our sample of 23 subjects are demonstrated. For each number of harmonics, maximal, minimal, and mean values of z over the 23 subjects are given. In the column farthest to the right the number of cases with z outside the 95% prediction area is displayed. The table shows that,

Table 2. Comparison of Modeled Transience Times to Results of Visual Inspection

	t_{up} (n=22)			t_{down} (n=15)		
	Mean±SD of difference (hours)	No. of differences ≤ 0:20 hours	biased towards	Mean±SD of difference (hours)	No. of differences ≤ 0:20 hours	biased towards
Square wave model	0:06 ± 0:35	21	-	0:01 ± 1:26	10	Later values
Segmentation based on a 4-harmonic Fourier model	-1:37 ± 1:35	4	Earlier values	0:45 ± 1:07	3	Later values
Segmentation based on a 8-harmonic Fourier model	-0:12 ± 0:54	10	-	-0:04 ± 1:30	7	-
Segmentation based on a 10-harmonic Fourier model	-0:25 ± 0:49	12	Earlier values	-0:15 ± 1:22	8	-

A positive difference indicates later model values. Bias towards later or earlier values was calculated from the sign of the differences (sign test with ties, $p<0.05$). t_{up} and t_{down}, times of transience to the high and low pressure period, respectively.

according to the first criterion, only Fourier models with 10 or 11 harmonics are adequate. According to the second criterion, models with eight up to 12 harmonics are acceptable. This is in contrast to the study presented by Chau, in which, according to the second criterion, four or five harmonics were sufficient to model circadian blood pressure variation.

A possible explanation for this difference may be that we used 20-minute averages of blood pressure, whereas the original study by Chau was based on one single measurement in each period of comparable length. To validate this hypothesis, we simulated single measurements from our data by a Monte Carlo experiment, and subsequently determined the number of harmonics needed in the model. This procedure was repeated 20 times.

Table 3. Sensitivity of Square Wave Parameter Estimation to the Use of Incidental Measurements

	t_{up} (hours)	t_{down} (hours)	BP_{high} (mmHg)	BP_{low} (mmHg)	BP_{mean} (mmHg)
Inaccuracy in the individual subject	-0:04	-0:05	1.26	-0.27	0.00
Imprecision in the individual subject	1:06	1:01	2.84	2.20	0.90
Imprecision in the group average	0:51	0:32	3.65	0.75	0.06

Inaccuracy, imprecision are represented by the mean and standard deviation, respectively, of the difference with values calculated from continuous measurement. BP_{mean}, average 24-hour mean arterial pressure; BP_{high}, BP_{low}, average mean arterial pressure observed during the high and low pressure period, respectively; t_{up} and t_{down}, times of transience to the high and low pressure period, respectively.

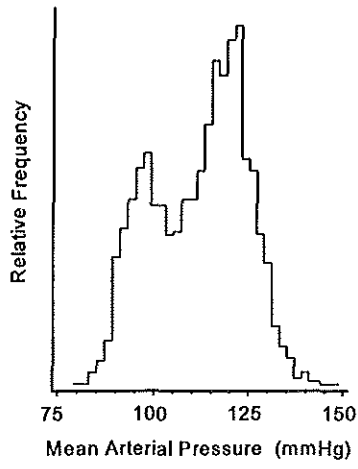

Figure 5. Histogram of standardized 24-hour mean arterial pressure, which illustrates the bimodal character of diurnal blood pressure profiles in our group.

According to the first criterion, five harmonics (15 cases) or six harmonics (five cases) were needed. The second criterion required three (three cases), four (four cases), five (eight cases) or six (five cases) harmonics in the Fourier model. It may be concluded that the use of single measurements instead of averages of continuous measurements reduces the number of harmonics that is needed for an adequate fit. This may be explained by the random variation between single blood pressure measurements that obscures systematic variation that remains from an inadequate fit. To establish the sensitivity of the method to the number of harmonics in the Fourier model, we performed calculations using a model of four, eight and 10 harmonics.

The average values for t_{up} and t_{down} obtained by Fourier modeling are displayed in Table 5. A comparison to values obtained by visual inspection is given in Table 2. As expected, the plausibility of results of the 4-harmonics model is poor, agreeing within 20 minutes with observer results in only four of 22 cases for t_{up} and in three of 15 cases for t_{down}. Both parameters are biased in such a way that, on the average, the duration of the low pressure period is underestimated by more than 2 hours as compared to the observers. Applying an eight-harmonics model gives a considerably larger number of plausible values, and the 10-harmonics model

Table 4. Results of Runs Test

Number of harmonics	z_{min}	z_{max}	z_{mean}	z_{SD}	No. of cases of $z>1.96$ or $z<-1.96$
1	-6.65	-2.35	-4.61	1.41	23
2	-4.48	-1.85	-3.09	0.73	21
3	-4.75	-1.42	-3.03	0.82	21
4	-4.27	-1.42	-2.81	0.90	18
5	-4.26	-0.90	-2.24	0.71	14
6	-2.50	-0.07	-1.41	0.73	5
7	-2.61	0.03	-1.33	0.70	4
8	-2.12	0.06	-0.90	0.86	1*
9	-2.13	0.95	-0.62	0.72	2*
10	-2.13	1.91	-0.20 †	0.98	1*
11	-1.85	1.67	0.32 †	0.86	0*
12	-1.42	2.61	0.64	0.92	1*

Minimum (z_{min}), maximum (z_{max}), mean (z_{mean}) and standard deviation (z_{SD}) of the z-values resulting from application of the runs-test to the residuals of harmonic analysis using 1 to 12 harmonics (n=23). *, $p>0.05$ (second criterion); †, $p>0.05$ (first criterion).

results in the largest number of plausible values for t_{up} and t_{down}, agreeing with observers in 12 of 22, and in eight of 15 cases, respectively (Figure 4B). For the 10-harmonics model, however, there is a tendency toward earlier values of t_{up} as compared with visual inspection ($p<0.05$). The number of plausible values obtained by any of the Fourier models is lower than the number obtained by square wave fitting. For t_{up}, the scatter of the differences with visual inspection is larger for the Fourier models than for the square wave model, as appears from the values of the standard deviation. For t_{down}, the scatter is comparable.

Mean±SD of blood pressure gradients obtained by Fourier models using four, eight and 10 harmonics over our group of 23 subjects were 7.4±4.1, 16.5±6.7 and 20.6±9.2 mmHg/hour for sl_{up} (see Appendix), and -7.5±3.6, -17.8±7.9 and -21.2±7.6 mmHg/hour for sl_{down}, respectively. Values obtained by the models with four and eight harmonics are smaller than those obtained by the model with 10 harmonics. Since the latter model provides a better fit to the original blood pressure profiles, we conclude that the four- and eight-harmonic models underestimate blood pressure gradients. The underestimation by the eight-harmonic model seems in conflict with the fact that adequacy of the fit has been established. Adequacy though is considered to be achieved if residuals of the fit of the entire 24-hour profile show no random positive or negative sequence. In periods of a shorter duration, e.g., at the sides of the trough, there may still be a systematic pattern in the residuals.

Quantitative Comparison of Methods

We calculated the percentage of total 24-hour variance in individual profiles that the various models in this study accounted for. Mean and standard deviation of these values

Table 5. Comparison of Models

Method	mean ± SD of PVA (%)	Number of parameters	t_{up} (h:m)	t_{down} (h:m)
Square wave	66 ± 16	3	7:05 ± 1:04	22:37 ± 1:43
Observer 1	58 ± 18	3	6:55 ± 0:53	22:52 ± 1:06
Observer 2	59 ± 17	3	6:49 ± 0:53	23:21 ± 0:45
Clock time	50 ± 18	1	(7:00 ± 0:00)	(23:00 ± 0:00)
Minnesota Cosinor Method	47 ± 19	2	–	–
Segmentation based on a 4-harmonics Fourier model	44 ± 22	3	5:13 ± 1:40	24:10 ± 1:27
Segmentation based on a 8-harmonics Fourier model	60 ± 18	3	6:38 ± 1:12	22:32 ± 1:41
Segmentation based on a 10-harmonics Fourier model	61 ± 19	3	6:24 ± 0.54	22:14 ± 1:40

PVA, percentage of 24-hour variance modeled; t_{up} and t_{down}, times of transience to the high and low pressure period, respectively.

are displayed in Table 5, together with the number of parameters of each model. When appropriate, the mean values of t_{up} and t_{down} as determined by the method are also displayed in the two right columns.

Discussion

Visual Inspection

The good correspondence between the two observers demonstrates that in most 24-hour registrations of heart rate and blood pressure, an evident and circumscript transience to a period of higher activity is visible. In contrast to the small interobserver variation, there was a considerable scatter in the indicated times of transience between profiles, which indicates that even in the synchronizing hospital environment, activity patterns as they appear from the registrations may vary considerably. Apparently, segmentation based on clock time does not do justice to the interindividual variation in the circadian variance of blood pressure.

On the other hand, it is evident that in a considerable number of blood pressure profiles, there is no evident single value for t_{down}. The observer bias for t_{down} that we have demonstrated, will cause segmentation of blood pressure profiles by visual inspection to be observer dependent. Therefore, it cannot be used for a reproducible assessment of the circadian blood pressure variation. Similar problems can be expected for t_{up} when the rise of blood pressure in the morning is substantially reduced, as is the case in various pathological states that diminish the circadian variance. Since the characterization of circadian variance is of most interest in these cases, this is also an important limitation to the use of visual inspection.

Square Wave Method

Our comparison clearly indicates that models of circadian blood pressure variation based on segmentation of the profile in a contiguous high and low blood pressure span may cover a considerable fraction of total 24-hour blood pressure variance. In this respect, the models based on segmentation compare favorably to the Minnesota Cosinor method. The model of segmentation is further supported by the bimodal distribution of blood pressure, which we demonstrated in this study. When the objective of blood pressure modeling is such a segmentation, the presented square wave fitting technique is optimal with respect to the square error. Moreover, since values for t_{up} and t_{down} are calculated to yield a best fit to a given profile, it is expected that the method yields valid results also when subject activities are not synchronized in time.

The time of passage to the high pressure span as determined by square wave fitting of mean arterial pressure compared well to results obtained by visual inspection using clock time, heart rate, and blood pressure information. When observers agreed about the time of passage to the low pressure span, square wave fitting resulted in a good or reasonable approximation of this time point. In many cases, however, there is apparently no evident single point in time of transience from the high to the low pressure span. Therefore, the interpretation of t_{down} as obtained by square wave fitting must be limited to its mathematical definition, i.e. the best fitting time of transience to the low blood pressure period. The method is conceptually simple, and its parameters are easy to interpret.

Application of the method needs no human interpretation, and therefore offers immediate and objective results.
In concordance with di Rienzo et al[17], we found that the estimation of mean 24-hour blood pressure can be based on incidental measurements. Also, the estimation of t_{up} and t_{down} by square wave fitting is unbiased. We conclude that the method can be applied in smaller data sets obtained in noninvasive ambulatory blood pressure measurements. An indication of the imprecision and inaccuracy of parameter estimation that results from the use of such material can be found in Table 3.

Clock Time
Surprisingly, modeling of circadian blood pressure variation by segmentation based on fixed clock time accounts for a large fraction of 24-hour variation when compared with some other, more sophisticated, methods in this study. Without fitting, more of circadian variation is described by the clock time model than by the cosinor model, even though the first model uses only one parameter whereas the latter needs two parameters. Two causes are responsible for this good performance. First, the period of rest and the period of activity are synchronized in the subjects of our study. Second, since the subjects did not work during the blood pressure registration, blood pressure during the period of activity was relatively constant. Segmentation based on fixed clock times is easy to perform, and the interpretation of its parameter, i.e., the difference of mean observed blood pressure during the two time periods, is straightforward. However, the description of circadian variation by this model is expected to be less adequate when activities are not synchronized.
Apart from common hospital activities, like meals and the times of night rest, no synchronization of activities was forced on the subjects in our group. Some subjects showed a marked lowering of blood pressure during the early evening to a level that was comparable to levels during the night, probably as a consequence of relaxation, while others maintained a rather constant level of blood pressure until the beginning of the period of night rest. For t_{up}, there was also a considerable scatter in the values. Modeling of blood pressure by fixed clock time periods cannot account for such differences.

Minnesota Cosinor Method
The cosinor method has been developed for the detection and quantification of biorhythms. When only the frequency of the rhythm is known, the use of a cosine is an obvious choice since harmonic analysis is a well-known tool of analysis of periodic phenomena and is theoretically well established. Also, it may well describe fluctuations that are sinusoidal, like the circadian fluctuation of cortisol level. Individual blood pressure profiles, however, do not have a sinusoidal shape; in particular the duration of the low blood pressure period is in general shorter than the duration of the high period, and the morning rise of blood pressure is abrupt instead of gradual. However, many authors have used this method for the characterization of circadian blood pressure variation. The fact that, in spite of these drawbacks, this method has been reported to give acceptable results is caused by summation of blood pressure profiles in a group of recordings. Since the abrupt rise of blood pressure during the morning will be at slightly different times in the various recordings, the sum profile will show a faint, elongated rise of blood pressure that resembles the ascending side of a sine, but that is not a

characteristic of individual blood pressure profiles. The cosinor method is therefore less appropriate to assess individual blood pressure profiles, and as expected, accounts for only a relatively small part of the actual circadian blood pressure variation. This is not only caused by the use of fewer parameters than the models that characterize circadian variation by segmentation, since it performs less well than clock time which uses only one parameter.

Segmentation based on Modeling with Multiple Harmonics

Similar to the square wave fit, the method presented by Chau et al[12] segments 24-hour blood pressure in a high and a low pressure span and specifies the times of transience between these spans. This segmentation accounts for a percentage of total 24-hour variation that is nearly as high as square wave modeling, which is optimal in this respect. In addition to the square wave fit parameters, blood pressure gradients at the transience times are calculated.

Various factors influence the validity of these blood pressure gradients sl_{up} and sl_{down}. First, their values depend on the number of harmonics in the model. The relevance of this number is stressed by the very poor performance of the four-harmonic model applied to our data and by the different blood pressure gradient values obtained by the various models. This number is dependent on the number of measurements in the profile.[18] Furthermore, it is dependent on the between-measurement variation, as we have demonstrated, and on the statistical criterion used to decide on this number. In our material, at least 10 harmonics were needed in the Fourier model. We could partially attribute the discrepancy to the use of single measurements in the former study, which introduces additional between-measurement variation. Furthermore, the subjects in the study by Chau et al[12] had 8-hour labor tasks during the registration. This may have caused a more sinusoidal form of individual blood pressure profiles, and such profiles may be described by fewer harmonics. As a consequence of the increased number of harmonics needed for the modeling of our material, a new criterion using visual identification of borders of the low pressure span was needed to determine times of transience between high and low pressure. This makes the procedure laborious and subjective.

Second, global criteria are used to decide on adequacy of the fit, whereas blood pressure gradients are a local property of the signal, for which the fit may not be adequate. If this is the case, estimation of blood pressure gradients sl_{up} and sl_{down} may be biased. For these reasons, blood pressure gradients obtained by this method are greatly influenced by the method of measurement and subsequent analysis, and it is not evident that the values presented so far are in fact mainly determined by the analyzed blood pressure profiles. The values should be interpreted with great care, and their use is limited by the incomparability between different measurement protocols. When estimation of blood pressure gradients is not among the purposes of blood pressure modeling, square wave fitting is a more objective and direct way of estimating t_{up}, t_{down}, BP_{high} and BP_{low}, and is optimal with respect to the least square error of the fit.

An interesting finding of the present study is the discrepancy between t_{up} as determined by visual inspection and square wave fitting on one hand and the Fourier model with 10 harmonics on the other hand. In a few cases, harmonic analysis resulted in values of t_{up} that were 2-4 hours earlier than the values determined by observers or by square wave

fitting. Since harmonic analysis indicates the largest gradient in the ascending track of blood pressure, we conclude that in these cases, the major part of the blood pressure rise is not at the time of the largest gradient, but occurs more gradually later in the morning. Since the gradient has been reported to be largest just after wakening, this suggests that segmentation based on the sleeping and wakening times is in some cases less appropriate for the modeling of circadian blood pressure variation.
The conclusions based on the current study are valid for hospitalized patients and probably hold for any population with a constant level of activity during the day. Additional research, however, is needed to assess the percentage of variation that the model accounts for in blood pressure profiles of outpatients.

Appendix - Conventional Methods of Modeling

Visual Inspection
Two independent observers, who were familiar with blood pressure registrations but not acquainted with the square wave fitting method, were asked to divide the 24-hour registrations of mean arterial pressure in two complementary periods of activity and rest. For this segmentation, the combined information of clock time and 24-hour graphs of heart rate and systolic, diastolic, and mean arterial blood pressure was used. Subsequently, the average of mean arterial blood pressure observed during both periods was calculated. In this way, the 24-hour blood pressure profile was modeled as two periods of constant pressure. Values of t_{up} and t_{down} were compared to detect observer bias. For this purpose, the sign of the difference between both observers was tested for random distribution at a significance level of 95% (sign test with ties[16]). Furthermore, mean values and standard deviations of the differences were calculated.

Segmentation by Clock Time
If the activity of a group of subjects is tightly coupled to the time of day, as is the case in a hospital environment, their activities will be synchronized rather strictly. In such a case clock time may be used to select periods of high and low blood pressure. We used the times of turning off the lights and of serving breakfast in the hospital (23:00 hour and 7:00 hour, respectively) to segment blood pressure profiles in two periods and modeled the 24-hour blood pressure profile using the average blood pressure during these two periods.

Minnesota Cosinor Method (One Harmonic)
This method enables the detection of a sinusoidal rhythm and the objective and quantitative determination of its amplitude and phase. It has been used by its authors[12,19] and others[20,21] to describe circadian blood pressure variation. Elaborate and critical reviews of the method are given by van Cauter and Huyberechts[22] and Bingham et al[23]. In short, the method fits a cosine with a period of 24 hours to a blood pressure profile. The phase of the fitted cosine is inverted and referred to as "acrophase". Amplitude and acrophase of the fitted cosine are subsequently used to describe circadian variation.

Segmentation Based on Modeling With Multiple Harmonics

The method described by Chau et al[12] quantifies the shape of a 24-hour blood pressure profile by Fourier modeling. The number of harmonics that is used in the model is determined by a statistical criterion. The modeled profile is not used to calculate circadian variance directly; instead, it is used to segment the original profile in a high and a low pressure period. This procedure thus defines a set of parameters identical to the square wave fit, which are used as characteristics of circadian blood pressure variation.

The segmentation is performed by identification of the minimum of the synthesized profile, followed by determination of the inflection points at both sides of this minimum (see Figure 6A for an example). These points are taken to be the points of transition

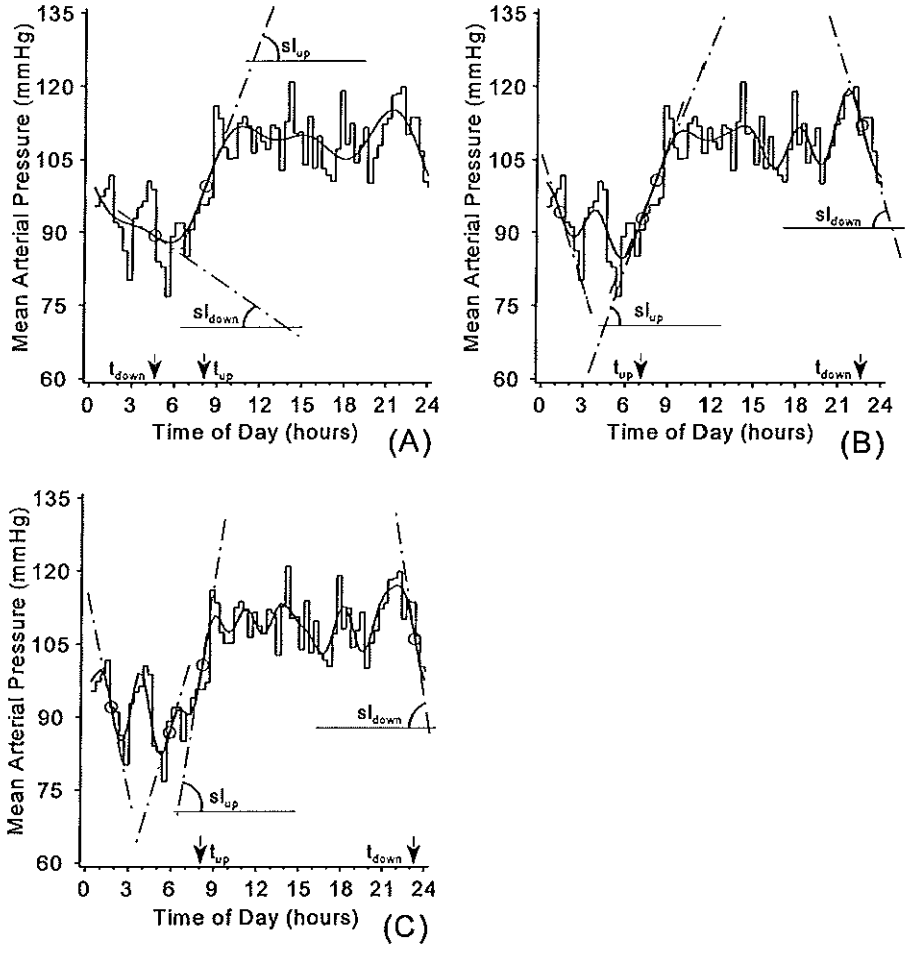

Figure 6. Modeling of circadian variation of blood pressure by harmonic analysis, using four, eight and 10 harmonic models. For the four-harmonic model (Panel A), inflection points in the model nearest to the minimum pressure (○) define t_{up} and t_{down}. This results in unrealistic values and a short modeled low pressure period. For the eight- and 10-harmonic models (Panels B and C), a relevant subset of inflection points is selected visually (○). From these, inflection points with the largest blood pressure gradients (indicated by sl_{up} and sl_{down}) define t_{up} and t_{down}.

between the period of low and of high blood pressure. In addition, the slopes in the modeled blood pressure profile at these points are determined. These parameters indicate the maximum blood pressure gradients of the synthesized function and serve as additional characteristics of circadian variance. We will refer to them as sl_{up} and sl_{down}, respectively. In this way, the method characterizes diurnal variation by a total of six parameters.

A fundamental part of the method is the determination of the number of harmonics needed for an adequate fit. Since the procedure for this determination is not explicitly described by Chau et al[12], it will be outlined here. A fit of a Fourier model to a single blood pressure profile is considered adequate if the distribution of positive and negative values of the residuals of the fit is random. To establish whether in a fit this criterion is fulfilled, the sequence of residuals in their chronological order is subdivided into alternating subsequences of positive and negative values. Such subsequences are called runs and for such runs a statistical test can be applied to verify a null hypothesis of random distribution[24]. In fact, the expectation value E(r) of the number of runs and the corresponding standard deviation S(r) under the null hypothesis are known. Thus, for a single fit yielding R runs, the test statistic

$$z = \frac{R - E(r)}{S(r)}$$

may be defined. This statistic is approximately standard normally distributed under the null hypothesis. This implies that each profile has a probability smaller than or equal to 0.05 to produce a z-value outside the range $-1.96<z<1.96$. In the original study by Chau et al[12], the mean z-value (z_{mean}) and the number of z-values out of the reference range were used to decide on adequacy of a Fourier model. According to their analysis, four harmonics were needed for an adequate fit of their blood pressure registrations. The exact criterion used for this decision, however, is not explained. For an objective comparison of our results to those obtained by Chau et al[12], an unambiguous definition of this criterion is required. For this purpose, we investigated two possible criteria.

When the z-values resulting from n independent fits are considered to be a sample of n elements from a standard normal distribution, it may be tested whether the mean value of z found for n fits is compatible with zero. The means calculated from a sample of n follow a normal distribution with (in this case) zero mean and a standard deviation of $1/\sqrt{n}$. Therefore, assuming a significance of 95%, harmonics should be added to the model until the mean value of z is in the range from $-1.96/\sqrt{n}$ to $1.96/\sqrt{n}$.

The study of Chau et al[12] uses a sample size of forty-five 24-hour blood pressure registrations (n=45), corresponding to a 95% prediction range for z_{mean} from -0.292 to 0.292. Using this criterion, none of the Fourier models presented in their study describes the systolic pressure adequately, and a model with five harmonics is adequate for diastolic pressure only. A different and more permissive criterion can be formulated using the probability levels of z-values resulting from individual fits. If the model is adequate, each profile has a probability smaller than or equal to 0.05 to produce a z-value outside the range $-1.96<z<1.96$. The 95% prediction interval for the maximum number of z-values out of this range in a sample of n elements is subsequently calculated assuming a binomial distribution. From n=45 it follows that only Fourier models that result in four or fewer

z-values out of the range $-1.96<z<1.96$ are acceptable at a significance level of 95%. For the data presented by Chau, this appears to be the case for both systolic and diastolic pressure if a Fourier model with 5 harmonics is used; a Fourier model with four harmonics is sufficient for diastolic pressure only. Since they concluded from their data that four harmonics offered an acceptable fit for both pressures, probably neither the first nor the second criterion has been used.

To establish the sensitivity of the method to the number of harmonics used, we performed calculations using a Fourier model with four harmonics as in the study by Chau, a model with 10 harmonics as is required by our first criterion, and a model with eight harmonics, which is sufficient according to the second criterion. The use of more than four harmonics, however, requires a modification of the original segmentation procedure. In the study by Chau, the limits of the low and high pressure span correspond to the inflection points in the synthesized blood pressure profile nearest to the minimum pressure (Figure 6A). The addition of more harmonics to the model, however, increases the number of inflection points, and therefore decreases their distance. As a consequence, a fit modeled by, for instance, eight harmonics produces low pressure spans with a duration of approximately two hours, which obviously is an unrealistic value. Therefore, we modified the algorithm of Chau et al as follows. Each 24-hour blood pressure profile in our sample included a large period of relatively low pressure, approximately during the period of rest. This period induced a minimum pressure in the modeled profile, and a surrounding trough. The sides of this trough were identified visually. Some of these sides appeared short and steep, and showed one inflection point, which was selected as a transition between the high and low pressure span. In some profiles, however, the transitions were gradual and elongated, and contained two or three inflection points, as is illustrated in Figure 6B and 6C. In such cases, the inflection point with the largest blood pressure gradient was chosen. This modified algorithm was applied when more than 4 harmonics were used in the Fourier model.

References

1. Pickering TG, Harshfield GA, Kleinert HD, Blank S, Laragh JH: Blood pressure during normal daily activities, sleep, and exercise. JAMA 1982;247:992-996
2. Mancia G, Ferrari A, Gregorini L, Parati G, Pomidossi G, Berinieri G, Grassi G, Di Rienzo M, Pedotti A, Zanchetti A: Blood pressure and heart rate variability in normotensive and hypertensive human beings. Circ Res 1983;53:96-104
3. Degaute JP, Van de Borne Ph, Linkowski P, Van Cauter E: Quantitative analysis of the 24hour blood pressure and heart rate patterns in young men. Hypertension 1991;18:199-210
4. Tilkian AG, Guilleminault C, Schroeder JS: Hemodynamics in sleep-induced apnea. Studies during wakefulness and sleep. Ann Intern Med 1976;85:714
5. Redman CWG, Beilin LJ, Bonnar J: Reversed diurnal blood pressure rhythm in hypertensive pregnancies. Clin Sci Mol Med 1976;51:687s-689s
6. Schalekamp MADH, Man in 't Veld AJ, Wenting GJ: What regulates whole body autoregulation. J Hypertens 1985;3:97-108
7. Shaw DB, Knapp MS, Davies DH: Variations in blood pressure in hypertensives during sleep. Lancet 1963;1:797-798

8. Littler WA, Honour AH. Direct arterial pressure, heart rate, and electrocardiogram in unrestricted patients before and after removal of a phaeochromocytoma. Am J Med 1979;53:441-449
9. Imai Y, Abe K, Sasakei S, Minami N, Nihei M, Munakata M, Murakami O, Matsue K, Sekino H, Miura Y, Yoshinaga K: Altered circadian blood pressure rhythm in patients with Cushing's syndrome. Hypertension 1988;12:11-19
10. Wenting GJ, van den Meiracker AH, Ritsema van Eck HJ, Simoons ML, Bos E, Weimar W, Man in 't Veld AJ, Schalekamp MADH: Lack of circadian variation of blood pressure after heart transplantation. J Hypertension 1986;4:578-580
11. Halberg F, Tong YL, Johnson EA: Circadian system phase. An aspect of biorhythms, in: Von Mayersbach H (ed): Cellular Aspects of Biorhythms. Berlin, Springer Verlag, 1967, pp 20-48
12. Chau NP, Mallion JM, de Gaudemaris R, Ruche E, Siche JP, Pelen, O, Mathern G: Twenty-four-hour blood pressure in shift workers. Circulation 1989;80:341-347
13. Millar-Craig MW, Hawes D, Whittington J: New system for recording ambulatory pressure in man. Med Biol Eng Comp 1978;16:727-731
14. Anderson TW: An Introduction to Multivariate Statistical Analysis. New York, John Wiley & Sons Inc, 1984
15. Albert A, Harrris EK: Multivariate Interpretation of Clinical Laboratory Data. New York, Marcel Dekker Inc, 1987
16. Siegel S: Nonparametric Statistics. International Student edition. Tokyo/New York, McGraw-Hill Book Co, Inc, 1956, pp 68-75
17. Di Rienzo M, Grassi G, Pedotti A, Mancia G: Continuous vs intermittent blood pressure measurements in estimating 24hour average blood pressure. Hypertension 1983;5:264-269
18. Streitberg B, Meyer-Sabellek W: Smoothing twenty-four-hour ambulatory blood pressure profiles: A comparison of alternative methods. J Hypertens 1990;8 (suppl 6):s21-s27
19. Halberg F, Drayer JIM, Cornélissen G, Weber MA: Cardiovascular reference data base for recognizing circadian mesor- and amplitude-hypertension in apparently healthy men. Chronobiologica 1984;11;275-298
20. Portaluppi F, Bagni B, degli Uberti E, Montanari L, Cavallini R, Trasforini G, Margutti A, Ferlini M, Zanella M, Parti M: Circadian rhythms of atrial natriuretic peptide, renin, aldosterone, cortisol, blood pressure and heart rate in normal and hypertensive subjects. J Hypertens 1990;8:85-95
21. Libretti A, Lattuada S, Rindi M, Grillo A, Salvaggio A: Temporal analysis of blood pressure by ambulatory 24 h blood pressure monitoring. Clin Exp Hypertens [A] 1985;7:473-467
22. Van Cauter E, Huyberechts S: Problems in the statistical analysis of biological time series: The cosinor test and the periodogram. J Interdiscipl Cycle Res 1973;4:41-57
23. Bingham C, Arbogast B, Guillaume GC, Lee JK, Halberg F: Inferential statistical methods for estimating and comparing cosinor parameters. Chronobiologica 1982;9:397-439
24. Siegel S: Nonparametric Statistics. International Student edition. Tokyo/New York, McGraw-Hill Book Co, Inc, 1956, pp 52-58

Chapter 5

On the Quantitative Analysis of 24-Hour Blood Pressure and Heart Rate Patterns

R.N. Idema, E.S. Gelsema, G.J. Wenting, and A.J. Man in 't Veld

Published in Hypertension 1992;19: 496-497 (letter)

Supported by a grant-in-aid for scientific research from the Netherlands Heart Foundation (NHS 86.070) and by the Cardiovascular Research Foundation Rotterdam

To the Editor:

In a recent article in *Hypertension*, Degaute and coworkers[1] applied the periodogram method as published by van Cauter in 1979[2] for the modeling of 24-hour profiles of blood pressure and heart rate. Although we agree with the authors that the objective description of such profiles is served by a mathematical model, the use of harmonic analysis for this purpose is not without hazards. A question of particular interest concerns the number of harmonics that should be used in the model, especially when the analysis is aimed at the extraction of particular form characteristics of the underlying waveform. Degaute et al[1], fitting at most three harmonics, postulated a bimodal form of 24-hour blood pressure and heart rate profiles. However, the adequacy of their fit should be established by a statistical criterion demonstrating random distribution of the fit's residuals. Surprisingly, Degaute et al[1] did not provide data relevant to this matter, although the original publication of van Cauter included two tests for this purpose.

In a recent study by Chau et al[3], also based on intermittent blood pressure measurements, at least four harmonics were needed to model circadian variation. Our own research on continuous blood pressure measurements[4] indicates a need for at least twice this number and explains the smaller numbers found in studies based on periodic (cuff) measurements by between-measurement variation. It seems therefore unlikely that conclusions on form

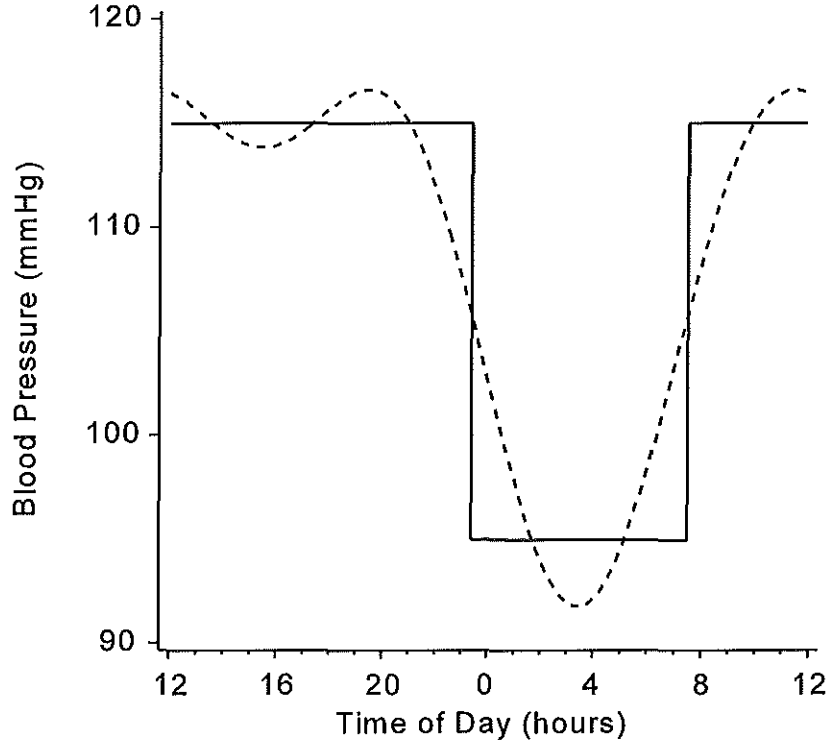

Figure 7. Line plot shows simulated blood pressure (solid line), modeled by the periodogram method as described by Degaute et al[1] (dashed line). The two maxima in modeled blood pressure do not represent the form of the actual blood pressure profile during the day; instead, they are induced by the nocturnal blood pressure fall.

characteristics of blood pressure profiles may be based on a three-harmonic model.
To demonstrate the pitfalls associated with harmonic modeling, we constructed an artificial 24-hour blood pressure profile, containing no variation except a pressure fall during sleep from 23:30h to 7:30h (solid line in Figure 1). Modeling with the described periodogram technique, using at most three harmonics, yields a bimodal profile (dashed line) with a profound nocturnal nadir at 3:20 hour and morning and evening acrophases at 11:30 hour and 19:20 hour, respectively. These results are very similar to those reported by Degaute et al[1] (3:39 hour for the nadir, and acrophases at 10:53 hour and 20:00 hour). Since in our simple example there is no variation whatsoever during the day, the morning and evening acrophases must be interpreted as modeling artifacts, induced by the nocturnal blood pressure fall. Indeed, looking at the profiles of, for example, systolic pressure in Figures 1 and 2 in the article by Degaute et al[1], these profiles may be as well interpreted as trimodal, with a third peak at approximately 14:00 hour. We conclude that the bimodality postulated by the authors is rather a consequence of the modeling technique than of blood pressure. Modeling artifacts such as those in this simple example may be detected by an examination of the residuals. If such an analysis is not performed, the quantification of form characteristics is not justified.
Also, we do not agree with the authors' statement that the reliability with which diurnal variation can be detected is dependent on the value of the amplitude expressed as a percentage of the 24-hour mean. For instance, adding a constant value to the measurements leaves the form of the profile intact and, as a consequence, the reliability of rhythm detection. However, this procedure diminishes the amplitude expressed as a percentage of 24-hour mean. In our opinion, only the modeled variance expressed as a percentage of total 24-hour variance is relevant for this purpose. Since values of modeled and total variance are not reported, we conclude that there is no indication in their article that diurnal variation is more reliably detected in diastolic than in systolic pressure.

References

1. Degaute J-P, van de Borne P, Linkowski P, van Cauter E: Quantitative analysis of the 24-hour blood pressure and heart rate patterns in young man. Hypertension 1991;18:199-210
2. Van Cauter E: Method for characterization of 24-h temporal variation of blood components. Am J Physiol 1979;237:E255-E264
3. Chau NP, Mallion JM, de Gaudemaris R, Ruche E, Siche JP, Pelen O, Mathern G: Twenty-four-hour ambulatory blood pressure in shift workers. Circulation 1989;80:341-347
4. Idema RN, Gelsema ES, Wenting GJ, Grashuis JL, van den Meiracker AH, Brouwer ML, Man in 't Veld AJ: A new method for diurnal blood pressure profiling - Square wave fit compared with conventional methods. Hypertension 1992;19:595-605

Chapter 6

Dependency of Square Wave Parameters on Measurement Frequency, Noise, and Nocturnal Blood Pressure Change

A Monte Carlo Experiment

R.N. Idema, A.J. Man in 't Veld, E.S. Gelsema

Submitted for publication

Introduction

In Chapter 4[1], the square wave fit was introduced for the modeling of the circadian variation of blood pressure. The method performed satisfactorily in profiles consisting of continuously recorded blood pressure in healthy subjects. The precision and the accuracy of the square wave fit parameter estimation, however, depend on the amplitude of the diurnal variation, and may therefore be decreased if the diurnal variation of blood pressure is attenuated. The parameter estimation is also dependent on the number of samples in the registration, and on the noise that is introduced in the signal by the measurement error and by blood pressure fluctuations. We assessed the dependency of the square wave fit on such variables by so-called Monte Carlo experiments. In these experiments, the square wave fit was applied to different square waves contaminated with noise. Precision and accuracy were assessed by comparing the parameters resulting from these fits to their predefined values.

Methods

Precision and Accuracy

The quality of a method of measuring is conveniently described in terms of precision and accuracy. Precision is a measure of reproducibility. It is by convention expressed as the standard deviation of repeated measurements of the same feature. Note that a small standard deviation indicates a high precision. The systematic error or bias of a measurement is defined as the average difference of repeated measurements and the true value. A positive bias indicates that the measurement overestimates the true value, whereas a negative bias incidcates an underestimation. Accuracy refers to the absence of bias, and hence a high accuracy implies a small absolute value of bias.

General Outline of the Monte Carlo Experiments

For the Monte Carlo experiments, a square wave model of diurnal blood pressure variation contaminated with normally distributed ("white") noise was assumed. In all experiments, the standard deviation of the noise was 8.0 mmHg throughout the 24 hours. This is approximately the value we have found in normotensive outpatients (see Chapter 7) after rejection of outliers, and corresponds to values reported by Berardi and co-workers[2] in non-invasive blood pressure measurements.

The precision and accuracy of the square wave fit parameter estimation are determined by the number of measurements in the profile, and by the signal to noise ratio (SN-ratio), i.e., the ratio of the variances contained in the square wave and in the noise. The number of samples in the profile is determined by the investigator who selects the frequency with which the blood pressure is measured, whereas the SN-ratio depends on the blood pressure pattern in the subject under study, and on the quality of the measurement. In the square

wave model assumed for the Monte Carlo experiments, the SN-ratio is determined by the ratio between the square wave amplitude and the standard deviation of the noise (AN-ratio), and by the lengths of the low and high period.

Monte Carlo experiments were performed for different sampling frequencies and for different lengths of the low and high period. In each experiment, the AN-ratio was varied in eleven steps between zero and 4.0. For each value of the AN-ratio, a large number (see below) of artificial 24-hour profiles contaminated with white noise was generated by means of SAS statistical software (SAS V6.04 for Personal Computers, SAS Institute Inc., Cary, North Carolina, USA). Subsequently, a square wave was fitted to each profile, and the square wave parameters amplitude and t_{up} were determined. These values were subsequently used to calculate the precision and accuracy of the square wave parameter estimation for the corresponding AN-ratio.

Dependency on Sampling Frequency

The dependency on the sampling frequency was assessed by fixing the length of the low period to 8 hours and by generating artificial profiles with a sampling frequency of 2.5 per hour and six per hour. The first value was chosen since most non-invasive ambulatory blood pressure profiles in our department are recorded with a frequency of approximately 2.5 measurements per hour, and the value of six measurements per hour was chosen since we consider this the highest measuring frequency that is acceptable to most subjects, although some authors have reported frequencies up to 8 measurements per hour[3]. In this way 300 profiles consisting of 60 sample in 24 hours, and 100 profiles consisting of 144 samples in 24 hours were generated.

Dependency on Length of Low and High Period

The dependency of the square wave fit on the length of the low and high period was assessed by fixing the sampling frequency at 2.5/hour and setting the length of the low period to four, eight, and 12 hours, respectively. For each length of the low period, 300 profiles were generated.

Amplitude

The accuracy of the amplitude estimation was calculated as the average difference of the amplitude from the predefined values. The precision was calculated as the standard deviation of this difference. When interpreting the values for precision it should be considered that the standard deviation of the amplitude estimation cannot decrease below the standard deviation SE of the difference between two "statistical" samples. If both samples have and equal standard deviation SD, as is the case in the present model, SE depends on the number of observations n_1 and n_2 in the two samples according to (1),

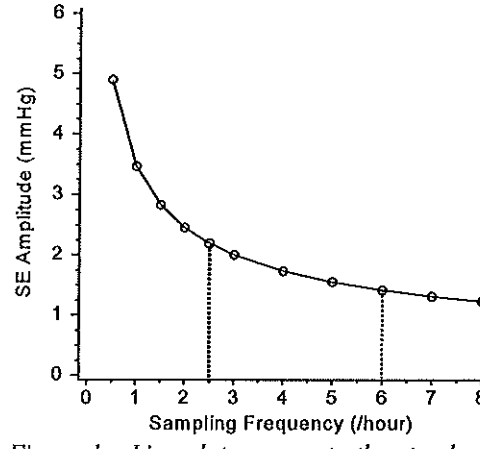

Figure 1. Line plot represents the standard error SE caused by the sampling error of the night-day difference of mean arterial pressure as a function of sampling frequency. Data are calculated for a standard deviation over the high and low period of 8.0 mmHg and a length of the low period of 8 hours. Dashed lines indicate the sampling frequencies used for the Monte Carlo experiments.

$$SE = SD\sqrt{\frac{1}{n_1} + \frac{1}{n_2}} \quad (1)$$

and therefore on the length of the low and high square wave period and on the sampling frequency (for an example see Figure 1). For a proper interpretation of the precision, the standard deviation of the square wave amplitude must be compared to this lower limit.

Transience Times

For the calculation of the statistics of clock time, a technique must be used that takes the circular aspect of these data into account. Methods that apply to such data have been developed for the analysis of directional data[5]. A description of these techniques is supplied in an Appendix. In short, clock time values are expressed as unit vectors with a length of 1.0 (by definition) and a direction that is given by the angle with zero on a 24-hour clock. Average and dispersion of a number of clock times are determined by calculating the vector average of the corresponding unit vectors, the so-called mean directional vector (MDV). The direction of the mean directional vector corresponds to the average time, whereas the length of the mean directional vector (MDVL) is a measure of dispersion. The range of the MDVL is between zero and 1.0. If all vectors have the same direction, the length of the average vector is 1.0, whereas the length is approximately zero if there is no preferential direction in the vectors. Therefore, the MDVL is inversely related to dispersion, and proportional to precision.

The precision and accuracy of the estimation of transience times was calculated as follows. The differences of the modeled values for t_{up} from the predefined values were expressed as unit vectors as described above. Subsequently, precision and bias of the estimation of t_{up} were calculated as the length and angle, respectively, of the vector average of these unit vectors. The calculations were performed for t_{up} only, since t_{down} has identical values for precision and opposite values for bias.

Significance of Square Wave Fit

The assumption that underlies the fitting of a square wave to a blood pressure profile is the presence of a square wave pattern, or a square wave-like pattern, in the blood pressure profile. The presence of such a pattern can be assessed by testing a null hypothesis H_0 that the square wave provides no better a description of the blood pressure profile than its simple mean. Thus, H_0 is rejected if the residual variance around the fitted square wave is significantly smaller than that around the 24-hour mean. The ratio between these two variances can be calculated directly from cc_{max}^2, since this parameter represents the fraction of the total variance that is modeled by the square wave fit. Under the null hypothesis, this ratio will follow an F-distribution after correction for the degrees of freedom, (one degree of freedom is lost with the estimation of the simple mean, and four degrees of freedom are lost with the estimation of the square wave parameters). Thus, for a profile consisting of n measurements, the F-ratio is calculated according to (2),

$$F = \frac{1}{1 - cc_{max}^2} * \frac{n - 4}{n - 1} \qquad (2)$$

and for statistical significance of the square wave fit at a level of $p<0.05$, one requires that $F>F_{0.05(n-1,n-4)}$.

In (2) it can be seen that the statistical power of this test is dependent on cc_{max} and therefore on the SN-ratio in the profile. Rejection of non-significant fits from a sample of blood pressure registrations may therefore be expected to attenuate the accuracy of, e.g., the amplitude of the square wave fit, since fits with a small AN-ratio will be preferentially rejected. The effect of rejecting non-significant fits was investigated by repeating the calculation of the precision and accuracy of the estimation of amplitude and t_{up} for significant fits only.

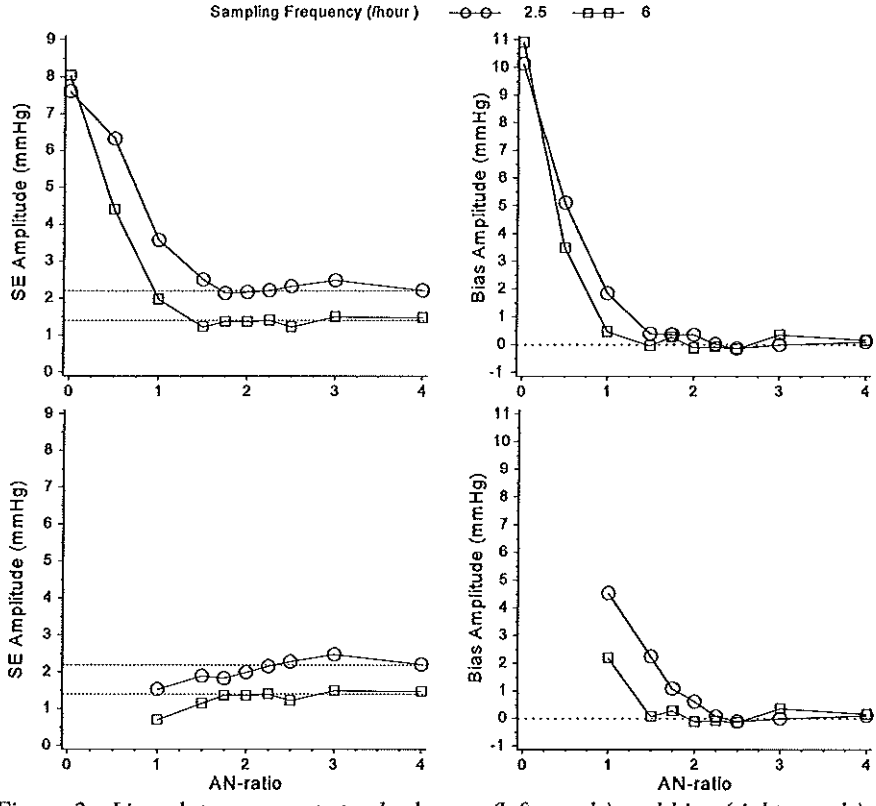

Figure 2. Line plots represent standard error (left panels) and bias (right panels) of square wave fit amplitude estimation as a function of amplitude to noise ratio for a measuring frequency of 2.5 and six measurements per hour. Data are calculated for a length of the low period of 8 hours and a standard deviation over the high and low period of 8.0 mmHg. Upper panel, all fits; lower panel, significant fits only. Horizontal dashed lines in left panels represent the standard error due to the sample size error for a sampling frequency of 2.5/hour (upper line) and 6/hour (lower line).

Results

Amplitude

The precision and accuracy of the square wave amplitude are depicted in the upper panels of Figure 2. As expected, the precision and accuracy increase with increasing AN-ratio. For AN-ratio's smaller than 1.0, the precision and accuracy sharply decrease. As expected, the precision and accuracy are better for the high sampling frequency than for the low sampling frequency. For an AN-ratio of zero, however, these values are independent of sampling frequency. In this case, only the noise is modeled, and the percentage of noise that can be modeled is independent of the sampling frequency. The positive bias of the amplitude estimation implies that some noise is modeled by the square wave fit.

If only the significant fits are considered (lower panels of Figure 2), the precision of the

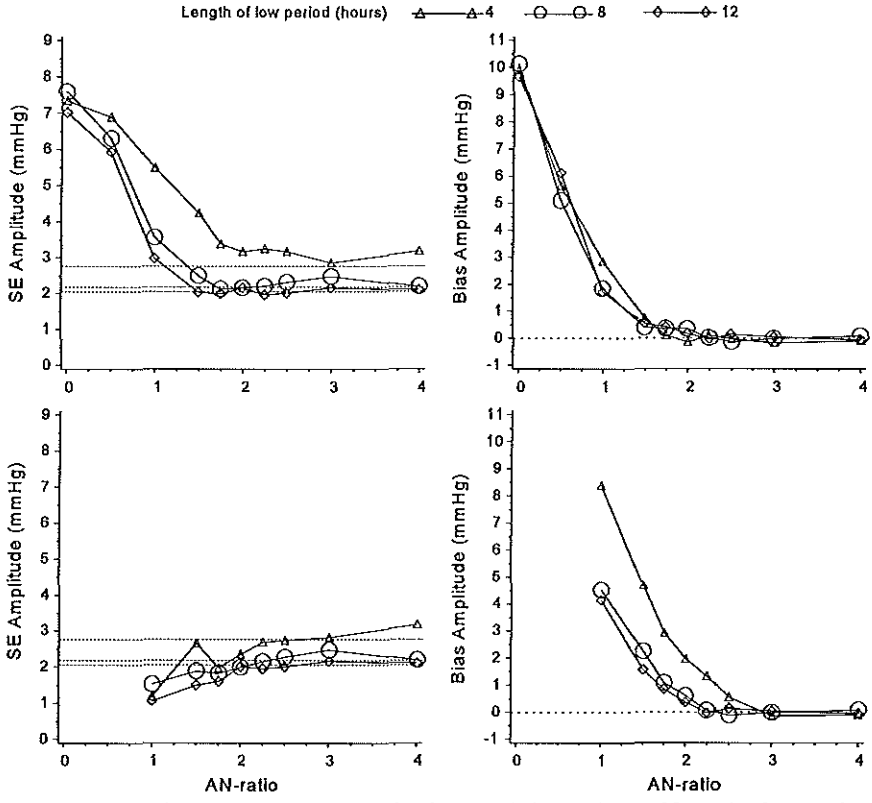

Figure 3. Line plots represent the standard error (left panels) and bias (right panels) of the square wave fit amplitude estimation for different lengths of the low period. Data are calculated for a sampling frequency of 2.5/hour and a standard deviation over the high and low period of 8.0 mmHg. Upper panel, all fits; lower panel, significant fits only. Horizontal dashed lines in left panels represent the standard error due to the sample size error for lengths of the low period of 4 hours (upper line), 8 hours (middle line), and 12 hours (lower line).

amplitude estimation appears to increase dramatically. In fact, the standard error decreases below the sample size induced standard error. The bias, however, increases, in particular for the low sampling frequency. These two phenomena can be explained by selection bias. Fits with a larger modeled amplitude tend to be more significant, and selection of significant fits will therefore preferentially exclude fits that have a small amplitude. This selection results in an increase of the average amplitude in the remaining fits, and in a decrease of the variation. Since the fraction of significant fits decreases with sampling frequency, the selection bias is most prominent for the low sampling frequency.

In Figure 3, the dependency of amplitude estimation on the length of the low period is depicted for a sampling frequency of 2.5/hour. The precision increases as the length of the low period approaches 12 hours. It can be easily verified that for a given amplitude, the variance in a square wave is maximal if the low and high period have an equal length, and that the SN-ratio is therefore optimal if the length of the low period is 12 hours. For the three square waves having lengths of the low period of 12 hours, 8 hours, and 4 hours, the SN-ratios are quantitatively related to each other as 9:8:5, respectively. This explains the

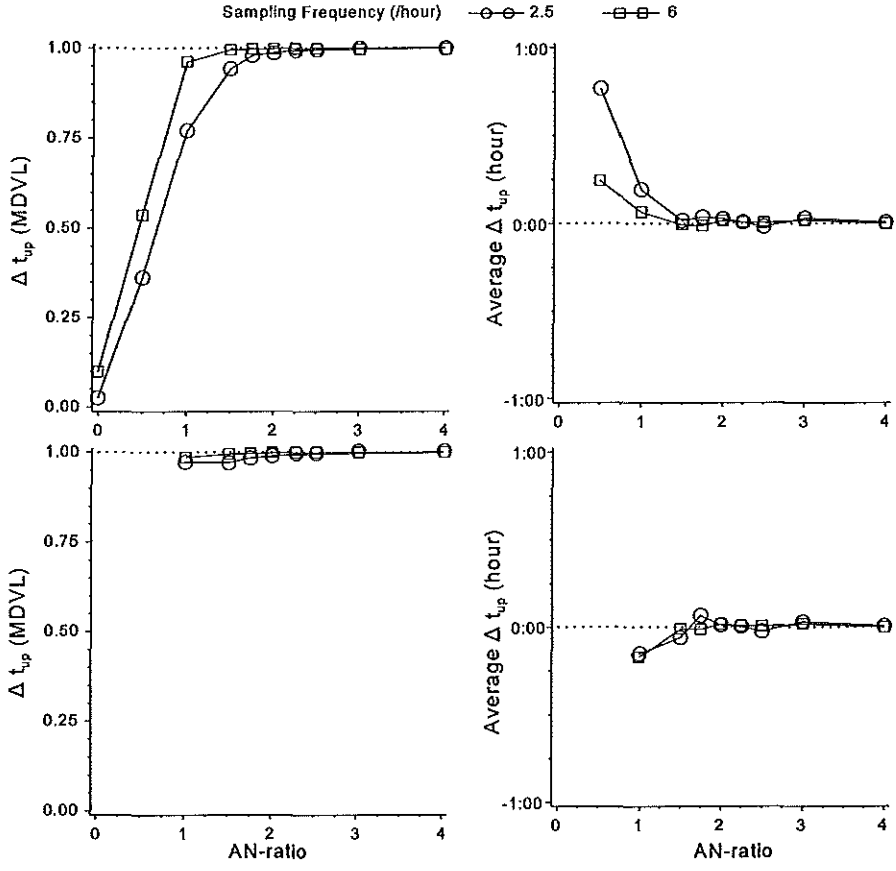

Figure 4. Line plots represent precision (left panels) and bias (right panels) of t_{up}-estimation as a function of the AN-ratio for different sample frequencies. Precision is represented by the length of the mean directional error vectors. Upper panels, all fits; lower panels, significant fits only. Δt_{up}, difference of modeled with predefined value of t_{up}.

increase of precision with increasing length of the low period. The bias of the square wave amplitude estimation, however, is insensitive to the length of the low period. If only significant fits are considered, the standard error decreases considerably at the cost of an increase of bias.

Transience Times

The standard error and bias of the estimation of t_{up} by square wave fitting are shown in Figure 4. As expected, the precision increases with increasing AN-ratio. For an AN-ratio larger than or equal to 1.0 (for 6 samples/hour) or 1.5 (for 2.5 samples/hour), the length of the average error vector is larger than 0.95, which corresponds to a standard deviation of the error of t_{up} smaller than approximately 1 hour. There is a slight positive bias smaller than 15 minutes if the AN-ratio is larger than 1.0. If only significant fits are considered, the precision is always larger than 0.95 and bias is at most 10 minutes. The square wave fit tends to model equal lengths of the low and high period, since this on average maximizes the fraction of noise that is modeled. In the present model the length of the low period (8 hours) is shorter than the length of the high period (16 hours), and therefore the length of the low period tends to be overestimated. This explains the small overestimation of t_{up}.

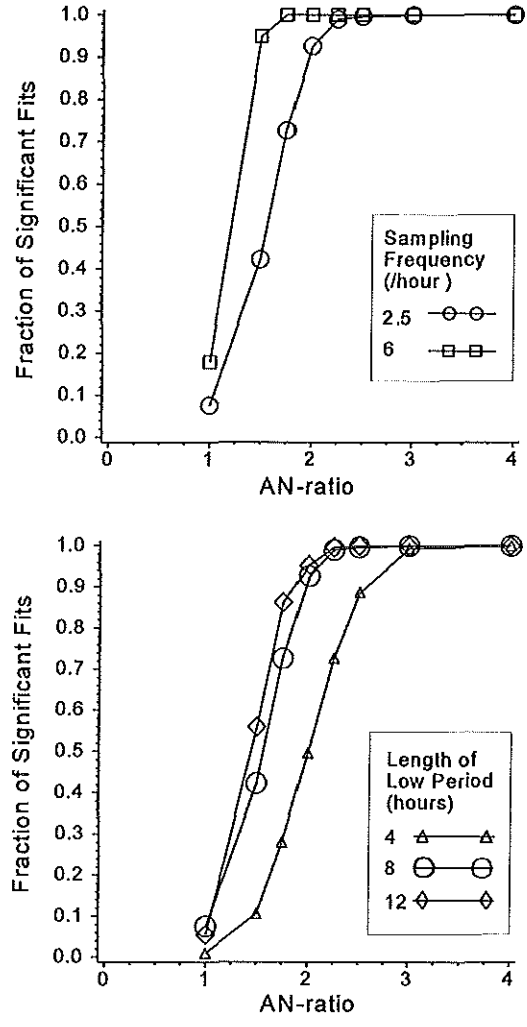

Figure 5. Line plots show the fraction of significant fits as a function of the amplitude to noise ratio. Upper panel demonstrates dependency on the sample frequency for a length of the low period of 8 hours. Lower panel demonstrates dependency on the length of the low period at a sample frequency of 2.5/hour.

Fraction of Significant Fits

In Figure 5, the fraction of square wave fits for which p is smaller than 0.05 is displayed. In order to attain a percentage of at least 90% significant fits, the AN-ratio should be at least 1.5 for a sampling frequency of 6/hour. For a sampling frequency of 2.5/hour, this ratio should be at least 2.0 (2.5 if the length of the low period is 4 hours).

Discussion

The results of the Monte Carlo experiment in the present study are in accordance with what is expected on theoretical grounds. The quality of the fit improves with an increasing sampling frequency, increasing amplitude of the square wave, and decreasing difference between the lengths of the high and the low period. The first phenomenon can be explained by decreasing sample error, and the last two phenomena can be explained by an increasing signal to noise ratio.

If blood pressure is measured six times an hour, and a length of the low period of eight hours is assumed, the square wave parameters can be reliably estimated if the amplitude is not smaller than the standard deviation of the noise. In such profiles, the mean±SD of the amplitude error are smaller than 0.5±2.0mmHg, whereas the sample standard error alone is 1.4 mmHg. The bias of t_{up} is a few minutes, with a mean directional error vector length of 0.96, which corresponds to a standard deviation of approximately 1.0 hour. About 20% of the fits have a p-value <0.05. If the AN-ratio is smaller than 1.0, the bias of the amplitude estimation increases to such an extent that the modeled amplitude will not decrease. In fact, if no restrictions are imposed on the fit, the average modeled amplitude will increase as the AN-ratio approaches zero. Since the underlying square wave disappears, it no longer restricts the phase and the length of the low period of the fitted square wave which thereby becomes "free" to optimally fit to the noise.
If blood pressure is measured 2.5 times an hour, the square wave parameter estimation is reliable if the AN-ratio is not smaller than 1.5. In such profiles, the mean±SD of the amplitude error are 0.5±2.5mmHg, whereas the sample standard error alone is 2.2 mmHg. In this case, the bias of t_{up} is a few minutes as well, with a mean directional error vector length of 0.96. About 40% of the fits have $p<0.05$. Since in healthy subjects the AN-ratio is approximately 1.5, blood pressure recordings should not consist of fewer than 2.5 measurements an hour. Preferably, the sampling frequency should be higher, since the AN-ratio will not be constant in the group, and the amplitude in the registrations with the lowest AN-ratios will be overestimated. In groups that may be expected to have an attenuated blood pressure rhythm, the blood pressure should be measured even more frequently. The necessity for an adequate number of samples is common to the square wave and other models of circadian variation. An alternative segmentation method described by Chau and co-workers[4] was applied in 24-hour profiles consisting of 96 measurements, whereas profiles that were modeled by the periodogram method by Degaute et al[3] consisted of 144 measurements.

As an example, consider a blood pressure profile recorded in a normotensive subject having a mean arterial pressure of 100 mmHg during the day-time, and of 85 mmHg during the nighttime. The profile is recorded with a frequency of 2.5 measurements per hour, and the lenght of the low pressure period is eight hours. Assuming a standard deviation of blood pressure of 8.0 mmHg, the AN-ratio is approximately 2.0. The square wave fit overestimates the amplitude in this profile by 0.4 mmHg (Figure 2, upper right panel). The sample size related imprecision of the amplitude is 2.2 mmHg, and the

additional imprecision induced by the square wave fit is negligible (Figure 2, upper left panel).
In a hypertensive subject having 25% higher values of the 24-hour average blood pressure, of the nocturnal decrease in blood pressure, and of the standard deviation, the AN-ratio is identical. Because of the larger standard deviation in this subject, the bias and imprecision are increased by 25% in comparision with the normotensive subject. The bias of the amplitude is therefore 0.5 mmHg, the sample size related imprecision is 2.8 mmHg, and the square wave induced imprecision is approximately 0.1 mmHg.

In order to establish whether the square wave fit can be used to assess the diurnal variation of blood pressure in an existing set of registrations, it must be established whether the AN-ratio in the profiles is sufficient for the given sampling frequency. If it is assumed that the group under study is homogeneous and that the length of the low pressure period is approximately 8 hours, the AN-ratio can be tentatively estimated from the fraction of significant fits in the group. This fraction must be at least 40% in profiles consisting of 60 samples, and at least 20% in profiles consisting of 144 samples. The values for different sampling frequencies can be derived from Figure 5.
The square wave amplitude can be used as a quantitative measure of the circadian variation of blood pressure only if the blood pressure rhythm is not severely attenuated, since the estimation is positively biased for small amplitudes. In cases of severe attenuation, the square wave fit can still be used to obtain qualitative information on the blood pressure rhythm. First, the estimation of t_{up} is less sensitive to the AN-ratio and may still be valid with the given AN-ratio. In this case, the distribution of this parameter over 24 hours may reveal differences between groups. Second, the fraction of significant fits in a group provides a qualititive measure of the diurnal variation in the group.
Exclusion of non-significant fits does not improve the assessment of the square wave amplitude since it increases amplitude-dependent bias. It can be used, however, to obtain a more reliable estimation of the transience times in the group.
In the current Monte Carlo experiment, the precision and accuracy of the square wave fit can be increased at will by increasing the sampling frequency. For blood pressure profiles, this is not possible. This is in part due to the practical limitations of cuff measurements, but these can be overcome by continous blood pressure measurement which generates a 24 hours profile consisting of more than 100,000 samples. A more fundamental problem is that the variation during the low and high pressure period in the blood pressure profile does not consist of white noise. Instead, the various fluctuations of blood pressure during the night and the day must be considered as an autocorrelated form of noise. Increasing the sampling frequency will therefore increase the information over the actual blood pressure during the high and low pressure period only to a limited extent.

Conclusion
In conclusion, this study confirms that the precision and accuracy of the square wave fit parameters are dependent on the measuring frequency, the length of the high and low period, and on the AN-ratio. In most healthy subjects, the amplitude of the diurnal variation is approximately 1.5 times the standard deviation over the day and night, and in such cases the square wave parameters can be reliably estimated in profiles consisting of 2.5 measurements per hour. If the amplitude is attenuated, but larger than the value of the

standard deviation over the day and night periods, the square wave fit can still be applied, but a higher measuring frequency is required. If the diurnal amplitude is smaller than the standard deviation, the square wave amplitude cannot be estimated reliably, but the transience times may still indicate the presence of a rhythm. In all cases, the percentage of square wave fits that are significant must be calculated to verify the validity of the method. The non-signficant fits should not be rejected since this introduces bias in the modeling.

Appendix - Analysis of Directional Data

Introduction

For an adequate analysis of clock time values, the circular character of these data must be taken into account. This can be intuitively understood by considering two clock time values t_1, 5 minutes before midnight, and t_2 , 5 minutes after midnight. These two values are almost identical and intuitively have an average value of 0:00 hour (midnight) and a standard deviation of a few minutes. However, if the values 23:55 hour and 0:05 hour are used to calculate the parameters of a normal distribution, an average of 12:00 hour (noon) and a standard deviation of 16:51 hour is found. This problem can be mitigated by expressing the values as differences with an estimated average, e.g. 0:01 hour, and by subsequently expresssing the values as the differences with the estimated mean, i.e. -0:06 hour and 0:04 hour, respectively. The mean±SD of these new values is 0:00±0:07 hour, which is better in accordance with expectation. For more dispersed values of clock time, however, this algorithm is of no value. For example, the average time of midnight and noon cannot be estimated in a rational way.

For the analysis of clock time values, methods can be used that have been developed for the analysis of directional data. The development of many of these techniques was originally inspired by problems in the Earth sciences[5], e.g., for the analysis of the orientation of geological characteristics in the landscape. According to these techniques, directional data are represented by unit vectors, and characteristics of the distribution of directional data are derived from these unit vectors.

Mean Vector Time and Dispersion

Clock times can be thought of as unit vectors, as can be understood by considering a unit vector as the hour hand of a clock with a 24-hour scale. The unit vector is represented by the angle θ with the X-axis. Since the zero point of clock time is at midnight (24:00 hour), which is by convention at 90 degrees with the positive X-axis, and the mathematical convention is to measure angles in the counterclockwise direction from the positive X-axis, clock time t is converted to θ by (3).

$$\theta = \frac{\pi}{2} - \frac{2\pi * t}{24} \qquad (3)$$

with θ expressed in radians and t in hours on a scale from 0:00 to 24:00 hour.

The dominant direction in a set of vectors can be found by computing the *resultant vector* R. The X and Y components of R for n unit vectors are calculated according to:

$$X_r = \sum_{i=1}^{n} \cos \theta_i$$
$$Y_r = \sum_{i=1}^{n} \sin \theta_i \qquad (4)$$

in which θ_i denotes the direction of the unit vectors. The X and Y coordinates of the *mean directional vector* $\underline{R}$ are calculated according to:

$$\underline{C} = \frac{X_r}{n}$$
$$\underline{S} = \frac{Y_r}{n} \qquad (5)$$

The direction $\underline{\theta}$ of the mean directional vector is calculated as the inverse tangent of the ratio of $\underline{S}$ and $\underline{C}$:

$$\underline{\theta} = \arctan\left(\frac{\underline{S}}{\underline{C}}\right) \qquad (6)$$

and the length of the mean directional vector $\underline{R}$ is calculated as:

$$\underline{R} = \sqrt{(\underline{C})^2 + (\underline{S})^2} \qquad (7)$$

The length of the mean resultant vector ranges from zero to one. It is a measure of dispersion, but expressed in the opposite sense, i.e. large values of $\underline{R}$ indicate that the observations are tightly bunched together with a small dispersion, while values of $\underline{R}$ near zero indicate that the vectors are widely dispersed. Other directional statistics can be computed, including circular analogs of the standard deviation, mode, and median. Equations for these are given by Gaile[6].
Circular distributions are conveniently described by the *von Mises distribution*, which is a circular variant of the normal distribution and is determined by the mean direction $\underline{\theta}$ and a concentration parameter κ. The von Mises distribution is unimodal and symmetric around the mean direction. As the concentration parameter increases, the likelihood of observing a directional measurement very close to the mean direction increases. If κ is equal to zero, all directions are equally probable, and the distribution becomes a circular uniform one.

It is difficult to determine κ directly, but the concentration parameter can be estimated from $\underline{R}$, if it is assumed that the data are a sample from a population having a von Mises distribution. Tables of κ calculated by maximum likelihood estimates from $\underline{R}$ are provided by Davis[5] and Mardia[7].

Test for Randomness

A simple hypothesis that can be tested is that the directional observations are random. In other words, there is no preferred direction, or the probability of occurrence is the same for all directions. If it is assumed that the observations come from a von Mises distribution, the hypothesis is equivalent to stating that the concentration parameter κ is equal to zero. The test is performed by calculating $\underline{R}$ according to (7). This statistic is compared to a tabulated critical value of $\underline{R}$ for the desired level of significance. If the computed statistic exceeds the critical tabulated value, the null hypothesis $\kappa=0$ is rejected and the observations may be presumed to come from a population having a preferred orientation. Critical values for $\underline{R}$ for various levels of significance are given by Davis[5] and Mardia[6]. The test can also be applied to the difference of paired observations. In this way, in analogy to, e.g., a paired t-test, the significance of the directional difference between pairs of observations can be assessed. The test for random orientation was originally developed by Lord Rayleigh at the turn of the century. A more recent description is given by Mardia[7].

Test for a Specified Trend

It may be of interest to test the hypothesis that the observations are compatible with a specific preferred direction. Exact tests of the hypothesis that a sample of observations has been taken from a population having a specified mean direction require the use of extensive charts in order to set critical values[8]. A simpler alternative is to estimate a confidence angle around the mean direction of the sample, and to verify if this angle is sufficiently large to encompass the hypothetical mean direction. Before computing the confidence angle, the data should be inspected or tested for compatibility with a von Mises distribution. Subsequently, the Rayleigh test should be applied to confirm that a statistically significant mean direction does exist. The approximate standard error of the mean direction, given in radians, is

$$SE = \frac{1}{\sqrt{n\underline{R}\kappa}} \tag{8}$$

Assuming that estimation errors are normally distributed, the confidence interval may be estimated as

$$\underline{\theta} \pm Z_{\alpha} SE \tag{9}$$

in which Z_{α} is the critical value for a standard normal distribution at a significance level α.

References

1. Idema RN, Gelsema ES, Wenting GJ, Grashuis JL, van den Meiracker AH, Brouwer ML, Man in 't Veld AJ: A new method for diurnal blood pressure profiling - square wave fit compared with conventional methods. Hypertension 1992:19:595-605
2. Berardi L, Chau NP, Chanudet X, Vilar J, Larroque P: Ambulatory blood pressure monitoring: A critical review of the current methods to handle outliers. J Hypertens 1992;10:1243-1248
3. Degaute J-P, Van de Borne P, Linkowski P, Van Cauter E: Quantitative analysis of the 24-hour blood pressure and heart rate patterns in young men. Hypertension 1991;18: 199-210
4. Chau NP, Mallion JM, de Gaudemaris R, Ruche E, Siche JP, Pelen, O, Mathern G: Twenty-four-hour blood pressure in shift workers. Circulation 1989;80:341-347
5. Davis JC: Statistics and data analysis in geology, 2nd edition. New York, John Wiley & Sons, Inc., 1986
6. Gaile GL, Burt JE: Directional statistics: Concepts and techniques in Modern Geography: 25 Geo Abstracts. Norwich: University East Anglia, 1980
8. Mardia KV: Statistics of directional data. London: Academic Press, Ltd., 1972
9. Stephens MA: Tests for randomness of directions against two circular alternatives. Jour American Statistical Association 1969:64:280-289

Chapter 7

Square Wave Fitting of Non-Invasive Blood Pressure Recorded in Normotensive Outpatients

R.N. Idema, A.H. van den Meiracker, E.S. Gelsema, A.J. Man in 't Veld

Introduction

In Chapter 4[1], a square wave model of circadian blood pressure variation was introduced and compared to existing models. The model was evaluated on profiles consisting of continuous intra-arterial blood pressure measurements recorded in subjects who were hospitalized in order to standardize environmental conditions. Intra-arterial recording, however, is an invasive method and it is not available to most investigators. Hospitalization is costly in terms of patient time and money and although it standardizes recording conditions, the parameters obtained in this condition may not be representative of the normal day to day blood pressure behaviour[2]. In the present study we therefore investigate the applicability of the square wave fit for modeling 24-hour profiles consisting of non-invasive measurements obtained in normotensive outpatients. Since the activities of these subjects were not synchronized, the relation between the transience times of the square wave model and the times of rising and going to bed will be analyzed in particular.

Table 1. Characteristics of Subjects and Blood Pressure Recordings

Subject	Age (y)	No. of Measurements	Average 24-hour Heart Rate (bpm)	Average 24-hour Mean Arterial Pressure (mmHg)	Time of Rise (hours)	Time of Going to Bed (hours)
1	48	63	74	101	7:45	23:00
2	45	54	67	87	10:45	0:00
3	34	60	63	89	8:15	23:00
4	31	60	91	90	4:45	22:00
5	45	61	76	94	9:36	1:30
6	47	48	70	90	7:30	0:00
7	44	54	79	105	8:15	23:15
8	35	57	72	93	7:30	0:30
9	48	61	81	117	6:10	22:00
10	53	56	83	89	8:45	23:30
11	45	55	74	94	6:40	23:00
12	43	56	60	109	7:20	23:30
13	42	61	80	101	6:33	23:50
14	31	49	63	88	11:00	1:45
15	43	55	70	98	8:50	22:45
16	36	58	75	91	6:00	23:00
17	51	63	68	102	5:30	22:00
mean	42	57	73	96	7:42	23:26
SD	6.7	4.4	8.1	8.4	1:39*	1:05*

* SD estimated from mean directional vector length (see (8) in Appendix of Chapter 6)

Subjects and Methods

Subjects

The blood pressure registrations for the present study were recorded during the placebo phase of a study on the effect of fluvastatine and gemfibrosil on vascular reactivity. Subjects for this study were recruited among male subjects with a combined hyperlipedemia (serum cholesterol 6.0-8.5 mmol/L and fasting serum triglycerides 2.5-8.0 mmol/L) who were 20 to 50 years of age. Smokers, subjects with signs of atherosclerosis, and subjects with a clinic systolic blood pressure larger than 165 mmHg or a diastolic pressure larger than 100 mmHg, were excluded. The subjects were otherwise healthy and did not use any antihypertensive medication. All subjects gave informed consent to participate in the study which was approved by the Medical Ethical Comittee of the University Hospital Dijkzigt.

Blood Pressure Registrations

Ambulatory blood pressure registrations were recorded by means of a Spacelabs ambulatory blood pressure measurement device (Spacelabs monitor model 90207, Spacelabs Inc. Redmond, WA, USA). Blood pressure was measured in the non-dominant arm. From

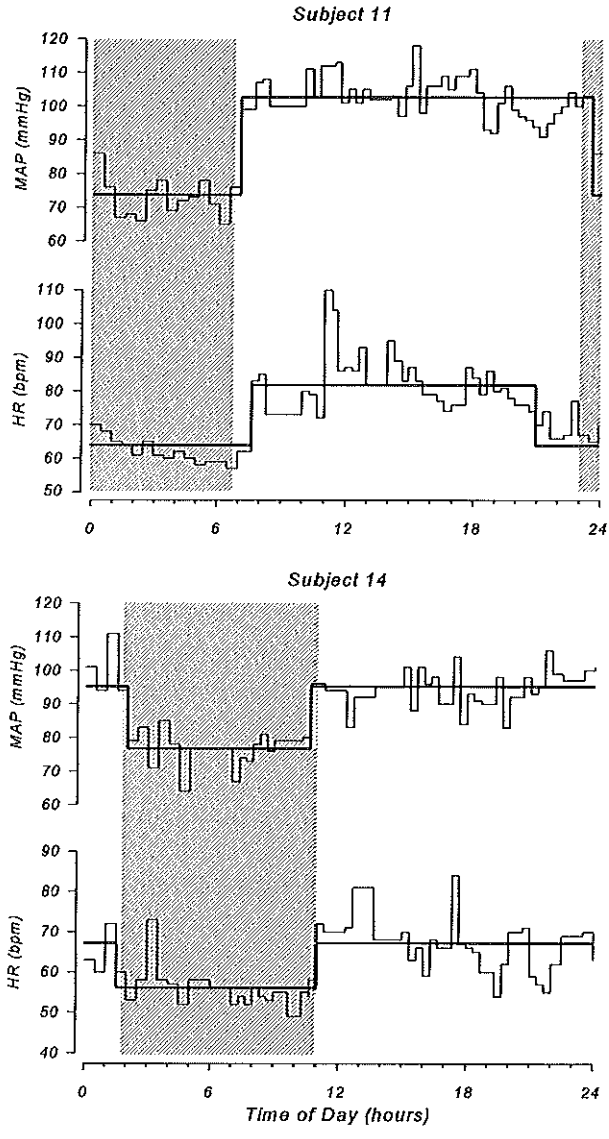

Figure 1. Profiles of mean arterial pressure and heart rate recorded in subjects 11 and 14 with fitted square waves (see ***Results*** *for explanation). The hatched area indicates the period of bed rest according to the diaries. Note that the period of bed rest and the square wave fit have different phases for the two subjects.*

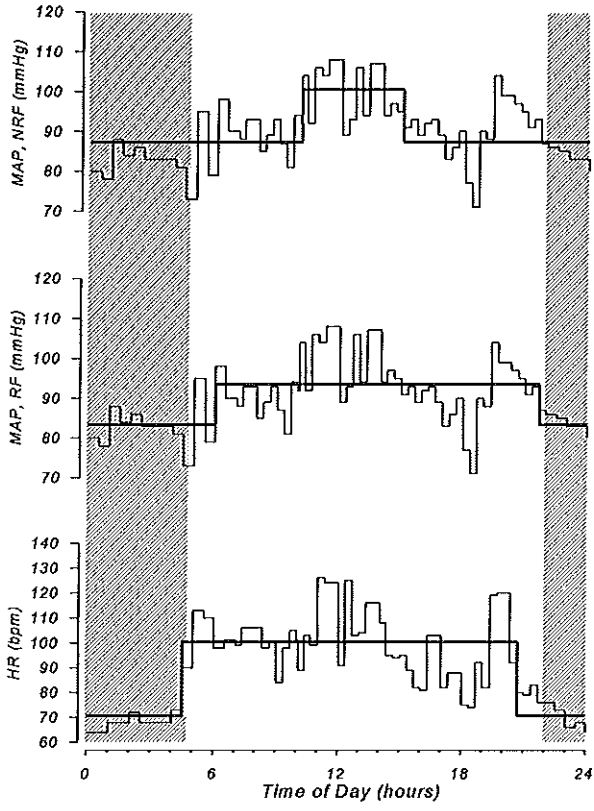

Figure 2. Mean arterial pressure (MAP) and heart rate (HR) recorded in subject 4 (see ***Results*** *for explanation). In the profile of mean arterial pressure, the square wave fit is determined by a short period of high pressure during the day rather than by the nocturnal decrease in blood pressure (upper panel). By restricting the possible length of the low period of the fitted square wave to a range of 5 to 13 hours (middle panel) the fit is determined by the circadian blood pressure variation. NRF, non-restricted fit; RF, restricted fit*

7:00 hour to 22:00 hour blood pressure was measured at 20 minute intervals and from 22:00 hour to 7:00 hour at 30 minute intervals (63 measurements). The subjects were advised to continue their daily activities during the recording period, but to be inactive and hang their arm by their side during each measurement. Apart from a visit to the cardiovascular laboratory to apply the blood pressure measuring device, the subjects were not restricted in their activities, and in particular were not instructed about the period of night rest. The subjects kept a diary in which they noted the time they went to and rose from the bed.

Outlier Analysis

Rejection of artifacts from the profiles was performed according to a method described by Parati et al[3]. This method estimates the range in which 95% of the measurements are contained as the mean±2SD, and rejects the values outside this interval. We applied the method to the combined profiles of heart rate and of systolic, diastolic, and mean arterial pressure, rejecting measurements in which at least one of these four parameters was outside the interval mean±2SD of the corresponding data distribution. The combined application of selection criteria, however, increase the rejection rate considerably (in our data to 18%). Therefore, the selection interval was extended to a range that contained approximately 95% of the observations (combination of four measurements). As the new selection interval the mean±2.5SD was chosen, and by this modified procedure, 5.7% of the measurements were rejected.

Nocturnal Change of Blood Pressure

The nocturnal change of blood pressure was calculated as the difference between the period of bed rest according to the diary and the complementary period. Arbitrarily,

subjects were classified as "dippers" if the nocturnal change of blood pressure was more than 10 mmHg and more than 10% of the 24-hour average pressure, and as "non-dippers" otherwise.

Square Wave Fitting

In Chapter 4, an algorithm was described for fitting square waves to profiles consisting of equidistantly spaced data. This algorithm was developed for the analysis of blood pressure profiles obtained by the Oxford technique[4]. This technique continuously measures 24-hour ambulatory intra-arterial blood pressure with little hindrance for the subject under study, and technical disturbances of the blood pressure recording are almost always of such a short duration that in most registrations a complete 24-hour profile of representative e.g. 20-minute averages can be calculated. If blood pressure is measured non-invasively, however, a compromise must be found between the frequency of measurements and hindrance of the subject. For this reason, blood pressure is often measured with a higher frequency during the day than during the night. An additional problem with cuff measurements is that in most cases some of the measurements will fail or will only succeed at a re-attempt a few minutes after the first measurement. Both mechanisms cause that blood pressure profiles obtained by non-invasive measurements most often consist of non-equidistantly spaced data. Since the present study is based on existing blood pressure recordings using such non-invasive measurements, the square wave fitting algorithm was modified. The non-equidistantly spaced character of these measurements was accomodated by using the time interval between measurements as a weighing factor in the calculations. Such a weighing is necessary if the measurements are not averaged over fixed time periods before they are used as input for the calculations, in order to correct for the fact that a period with

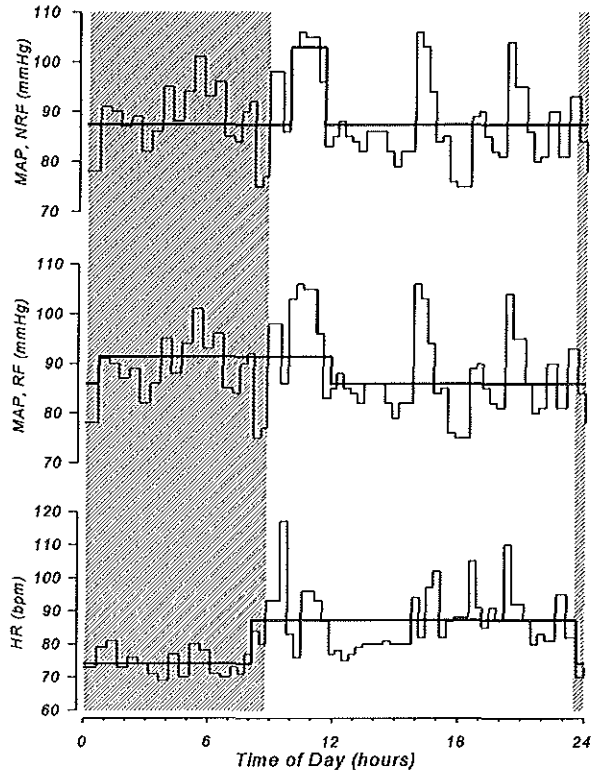

Figure 3. Profile of mean arterial pressure and heart rate recorded in subject 10 (see ***Results*** *for explanation). Although there is a normal circadian variation of heart rate (lower panel), there is no apparent pattern of circadian blood pressure variation, and as a consequence, restriction of the square wave fit (middle panel) does not improve the modeling of the circadian blood pressure variation in comparison with unrestricted square wave fitting (upper panel).*

a high sampling frequency, e.g., the day, contributes with more measurements to parameter calculations, e.g., the 24-hour average.

Fitting a Square Wave to Unevenly Spaced Data

To find the best fitting square wave for a given profile, cross correlations of the profile with all possible different square waves are calculated. The best fitting square wave is identified by the highest cross correlation coefficient. The square waves are generated as discrete functions, only defined at the time points of the actual measurements. For a profile consisting of N measurements, N*(N-1) square waves exist. This can easily be verified by considering that the low pressure period consists of at least one measurement and at most of N-1 measurements, and that for each of these alternatives, there are N different possibilities for the phase of the square wave.

As a first step in the modified fitting procedure, the profile P that is to be analyzed and each square wave are standardized to an average level of zero and a standard deviation of 1.0. The standardization is performed by calculating the weighted mean and weighted standard deviation over the profile, weighing each measurement with the length of the time period until the next measurement. The weighted mean is subtracted from the individual measurement values, and the resulting differences are divided by the weighted standard deviation. Subsequently, for each possible square wave the cross correlation coefficient cc is calculated as the weighted average cross product of the N corresponding values of the square wave and the blood pressure profile. The highest cross correlation coefficient, cc_{max}, identifies the best fitting square wave. The transience times of the square wave to the high and low period, t_{up} and t_{down}, respectively, are used to segment the original blood pressure profile in a high and low period. The low and high level of the modeled square wave, P_{low} and P_{high}, respectively, are computed as the weighted average of the original profile over these two periods. The amplitude of the square wave is calculated as the difference P_{high}-P_{low}.

The procedure described above specifies the general principle of fitting by cross correlation. Since, however, a square wave is a simple waveform, the calculations for the fitting procedure can be considerably simplified. First, the low and high levels of the square waves which are to be fitted can be calculated directly. Since the standardized square wave has an average value of zero and a standard deviation of 1.0, it follows that the values of the low level (SW_{low}) and high level (SW_{high}) of the standardized square wave are determined by the duration of these periods only, and can be calculated from, e.g., the length of the low period L according to (1), in which L is expressed in hours on a scale from zero to 24 hours.

$$SW_{low} = -\sqrt{\frac{24 - L}{L}}$$

$$SW_{high} = \sqrt{\frac{L}{24 - L}} \qquad (1)$$

The square waves that are fitted consist of two constant levels, and therefore the calculation of the average cross product, which employs N multiplications, can be reduced to calculating the weighted average of two cross products, i.e. the cross products of SW_{low} and of SW_{high} with the corresponding weighted average levels of the profile, weighing the cross products with L and 24-L, respectively. The number of calculations can be further decreased by calculating only the average value over the profile that corresponds to, e.g., the low level of the square wave that is to be fitted, and by deriving the average level that corresponds to the high level from this value. It can be easily demonstrated that for each square wave to be fitted, the cross correlation coefficient can be calculated directly from the length of the low period and the weighted average level S_{low} of the corresponding period of the standardized profile according to (2).

$$cc = -S_{low} * \sqrt{\frac{L}{24 - L}} \qquad (2)$$

While the different square waves are fitted, S_{low} can be calculated recursively from previous values in order to further reduce the number of numerical operations.

Statistics of Clock Time

For the calculation of the statistics of clock time, a technique must be used that takes the circular aspect of these data into account. Methods that apply to such data have been developed for the analysis of directional data[5]. A description of these techniques is supplied in the Appendix of Chapter 6. In short, clock time values are expressed as unit vectors with a direction that is given by the angle with zero on a 24-hour clock. Average and dispersion of clock times are determined by calculating the vector average of the unit vectors. The direction of the average vector corresponds to the average time, whereas the length of the average vector is inversely related to dispersion.

Conventional Statistics

Clock time statistics were calculated by means of techniques for the analysis of directional data. In addition, classical mean and standard deviations were calculated as an illustration of the difference between the two techniques. Correlation of clock times with diary times was assessed by the Spearman rank correlation coefficient. Before the correlation calculations, the clock times were expressed as the deviation from their median value. The purpose of this procedure was to correct for the circular aspect of the clock times and to express the difference between, e.g., the clock times 23:59 hour and 0:01 hour as 2 minutes rather than as 23 hours and 58 minutes. For parameters other than clock time, normal distribution was tested by the Shapiro Wilk statistic[6], and the significance of difference from zero was assessed using paired or unpaired t-tests for normally distributed parameters and by a signed rank tests[7] otherwise. For the assessment of statistical significance, a 0.05 significance level was chosen. All statistical computations were perfomed with SAS statistical software (SAS V6.04 for Personal Computers, SAS Institute Inc., Cary, North Carolina, USA).

Results

Subjects and Blood Pressure Registrations

Seventeen male subjects aged 42±6.7 yr participated in the study. The clinical characteristics of these subjects are listed in Table 1. All subjects were awake from 11:00 to 22:00 hour, and all subjects were asleep from 1:45 to 4:45 hour. The registrations appeared to be of sufficient quality. Of all scheduled measurements, 95.7% succeeded, and after the removal of outlying measurements (5.7%), an average of 57±4.4 measurements remained in the profiles.

Square Wave Fitting

In most profiles of heart rate and mean arterial pressure, the diurnal variation was modeled adequately by the square wave model, as was judged by visual inspection, and the fits appeared to follow the different periods of night rest in most subjects. Figure 1 depicts two of such square wave fits in profiles of mean arterial pressure and heart rate. In some profiles, however, although a normal pattern of diurnal rhythm was present, the square wave fit appeared to be determined by a period of high blood pressure or heart rate during the day and not by the nocturnal blood pressure fall. An example of such a profile is given in Figure 2.

We did not encounter this problem in square wave modeling of continuously measured blood pressure in hospitalized subjects[1]. The phenomenon has been encountered before, however, in a study employing non-invasively measured blood pressure by Fagard and co-workers[8]. This investigator improved the fitting by restricting the length of the low period. We adopted this modification, restricting the length of the low period to a range from 5 to 13 hours (*restricted square wave fit*). These values correspond to the range we reported in a study based on continuously measured blood pressure[1].

This modification was evaluated by visual inspection of the profiles and of the square waves calculated by restricted and non-restricted fitting. If a difference between the fits occurred, it was classified as an *improvement*, an *indifferent change*, or a *deterioration*. A change was classified as an improvement if the restriction imposed on the fit improved the description of the diurnal variation. An improvement occurred in profiles in which, despite a normal circadian variation, the unrestricted fit was determined by a short period of high blood pressure or heart rate during the day, as was the case in the example of Figure 2. A change was classified as indifferent if it did neither improve nor deteriorate the modeling of the diurnal rhythm as judged by visual inspection. Most changes classified as indifferent occurred in profiles in which no apparent diurnal rhytm was

Table 2. Adequacy of the Description of Diurnal Variation by Restricted vs Non-restricted Square Wave Fitting

	No Change	Indifferent Change	Improvement	Deterioration	Total
Heart rate	11	3	3	0	17
Mean arterial pressure	10	1	6	0	17

Changes are classified by visual comparison of restricted to non-restricted square waves fits

Table 3. Group Statistics of Diurnal Variation

	HR	MAP
Amplitude		
Restricted	17.0±7.9 bpm	15.7±7.2 mmHg
Non-restricted	18.4±7.6 bpm	17.9±6.1 mmHg
cc_{max}		
Restricted	0.67±0.12	0.67±0.17
Non-restricted	0.69±0.10	0.71±0.12
No. of Significant Fits		
Restricted	11	11
Non-restricted	13	12
t_{up} (h:m) [MDVL]		
Restricted	8:19 [0.76]*	7:32 [0.85]*
Non-restricted	7:38 [0.63]*	8:30 [0.86]*
t_{down} (h:m) [MDVL]		
Restricted	22:51 [0.78]*	22:39 [0.71]*
Non-restricted	21:52 [0.75]*	22:25 [0.45]*

Values denote mean±SD or, for clock times, the mean directional vector time and mean directional vector length (MDVL). bpm, beats per minute; *, $p<0.05$

detectable. An example of such a profile is given in Figure 3.

In none of the cases, the modeling of diurnal variation deteriorated by restricting the length of the low period. The results of the comparison of restricted and non-restricted square wave fitting are summarized in Table 2.

Square Wave Parameters

The parameters calculated from the 24-hour profiles of heart rate and mean arterial pressure are summarized in Table 3. The square wave amplitude appeared to be independent of 24-hour average blood pressure

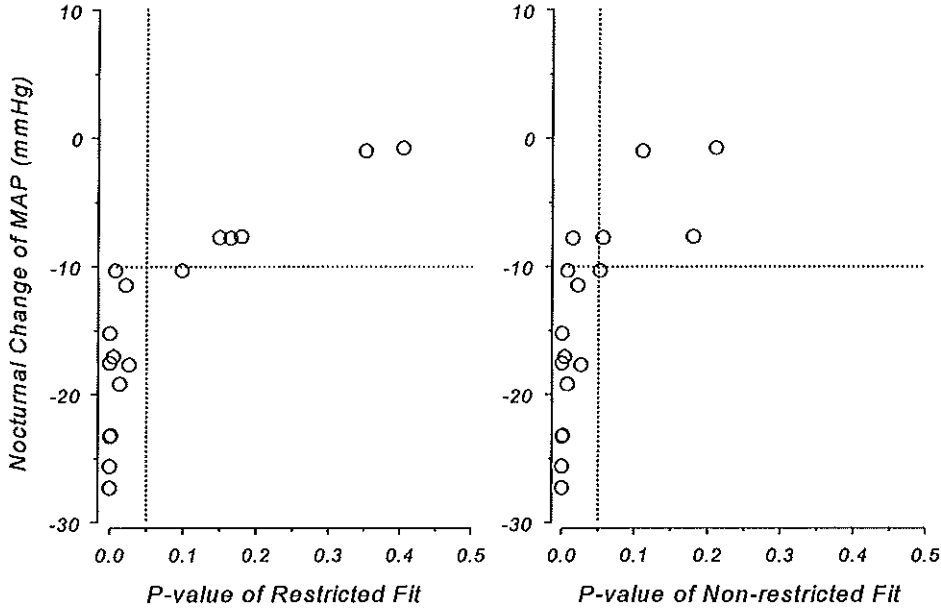

Figure 4. Scatter plot shows nocturnal change of mean arterial pressure (MAP) according to diary vs. p-value of restricted and non-restricted square wave fits.

(data not shown), and therefore the absolute value of this parameter is displayed rather than a percentage of the 24-hour average. The number of significant fits and the values for the amplitude and the cross correlation coefficient are larger for non-restricted fits. Such differences are expected since the non-restricted fit allows inclusion of a larger fraction of the total variance in the square wave model. This explains the larger values for cc_{max} and the larger number of significant fits, and a tendency to larger values for the amplitude. The values for t_{up} and t_{down} will be discussed in the next section.

Figure 4 depicts the relation between the decrease in mean arterial blood pressure during the period of night rest as indicated in the diary, and the p-value of the square wave fit. A nocturnal decrease in blood pressure larger than approximately 10 mmHg tends to give a significant fit whereas a less prominent nocturnal blood pressure change tends to give a non-significant fit, in particular in restricted fits.

Comparison of Transience Times to Diary Times

In Figure 5, the individual values of t_{up} and t_{down} are compared to times of rising from and going to bed as recorded in the diaries. The differences of transience times with the diary times are summarized in Table 4 and in Figure 6. For both heart rate and mean arterial pressure, the differences with the diary times are small, and reach statistical significance only for t_{down} modeled by means of non-restricted fitting in the heart rate profiles. There is a tendency to larger values for t_{up} and smaller values for t_{down} in comparison with the diary times, and hence a tendency to a longer period of modeled low blood pressure and heart rate than the period of bed rest. This pattern is more pronounced with non-restricted fitting than with restricted fitting. In addition, the non-restricted fit tends to give more variation in the differences with the diary times. The average deviation of t_{down} from the time of going to bed and the scatter of this deviation are much

Table 4. Deviations of Transience Times from Diary Times

	HR	MAP
t_{up}, restricted fit		
Δ MDVT (h:m) [MDVL]	0:38 [0.86]	-0:06 [0.89]
Δ mean±SD (h:m)	0:51±2:17	-0:22±2:08
Correlation with diary time of rise	0.65*	0.77†
t_{up}, non-restricted fit		
Δ MDVT (h:m) [MDVL]	0:03 [0.74]	0:48 [0.91]
Δ mean±SD (h:m)	-0:51±3:39	0:49±1:47*
Correlation with diary time of rise	0.71*	0.58‡
t_{down}, restricted fit		
Δ MDVT (h:m) [MDVL]	-0:37 [0.86]	-0:52 [0.75]
Δ mean±SD (h:m)	-0:30±2:13	1:34±3:24
Correlation with diary time of going to bed	0.35	0.59‡
t_{down}, non-restricted fit		
Δ MDVT (h:m) [MDVL]	-1:41 [0.84]‡	-1:22 [0.49]
Δ mean±SD (h:m)	-1:48±2:21*	-1.31±5:22
Correlation with diary time of going to bed	0.28	0.69*

Values represent mean directional vector times (MDVT) and -lengths (MDVL) in 17 subjects. Mean±SD are included in the table as an illustration of the difference between the two statistical techniques and do not represent study results.
Δ, difference of modeled transience times with diary times;
*p<0.01, †p<0.001, ‡p<0.05 for differences with zero

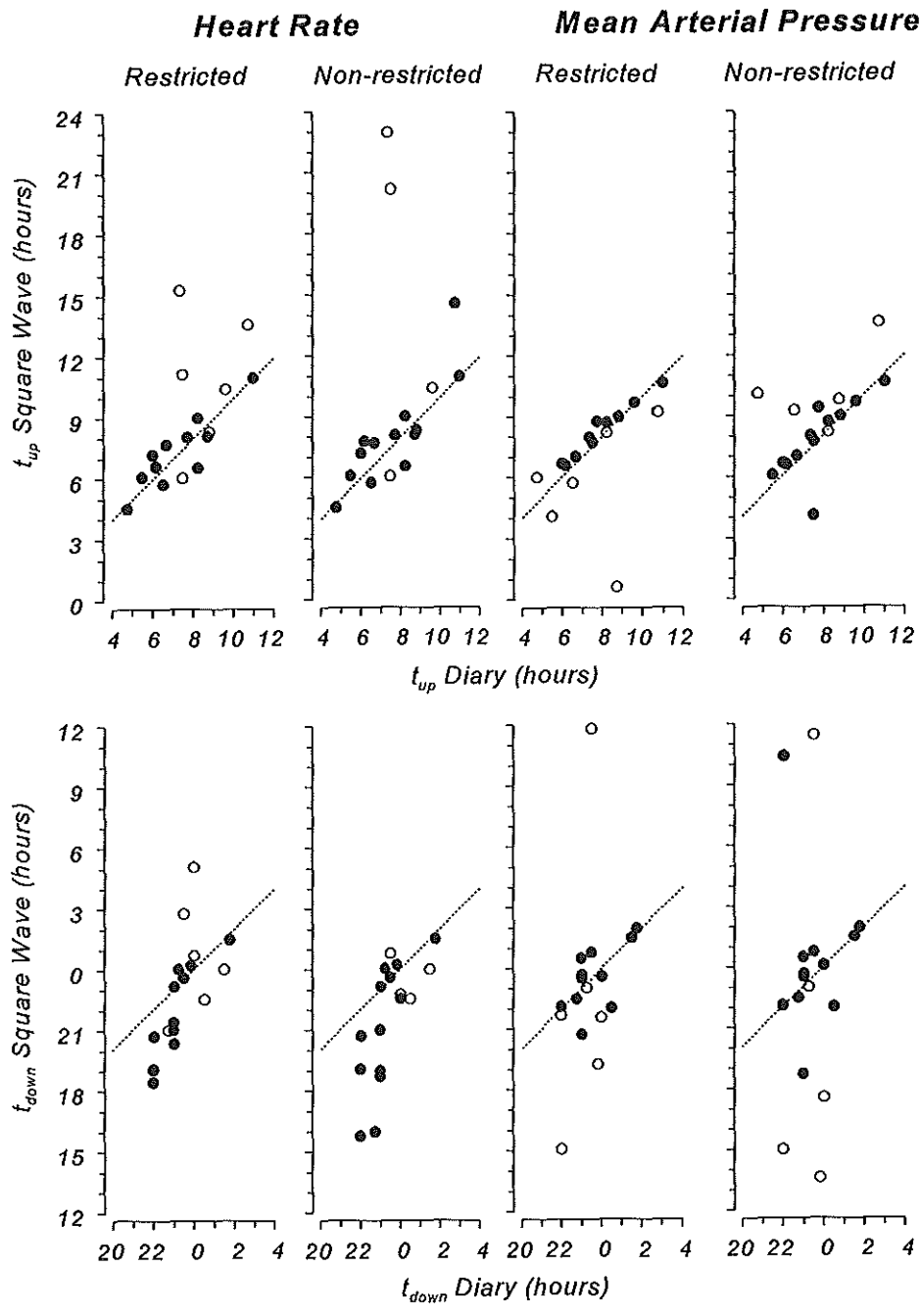

Figure 5. Comparison with diary times of values of t_{up} (upper plots) and t_{down} (lower plots) calculated in profiles of heart rate (left) and mean arterial pressure (right) by means of restricted (columns 1 and 3) and non-restricted (columns 2 and 4) square wave fitting. ●, fits with $p<0.05$; ○, fits with $p\geq0.05$; dashed line is line of identity.

larger than the deviation of t_{up} from the time of rise and the corresponding variation. For all parameters, the differences of transience times with diary times are smaller for significant fits than for non-significant fits. For mean arterial pressure, the transience times are correlated with the corresponding diary times, i.e., later times of rise and going to bed are associated with later values for t_{up} and t_{down}, respectively. For heart rate, the correlation is only significant for t_{up}.

Figure 7 depicts the deviations of t_{up} from the diary time of rise in relation to the the cross correlation coefficients in the individual profiles of mean arterial pressure. The smallest deviations appear to coincide with the largest cross correlation coefficients. The deviations from the diary times are smallest in restricted fits. Of the 17 subjects, 12 were "dippers". As expected, these "dippers" have the largest cross correlation coefficient values and the smallest deviations of t_{up} from the diary times. In 11 of the 12 "dippers", the square wave fit was significant.

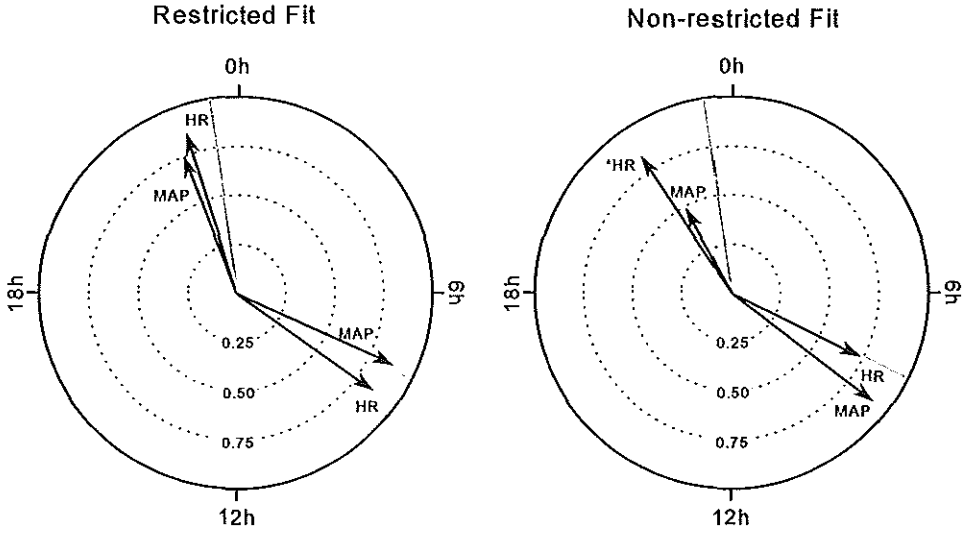

*Figure 6. Circular plots show average diary times of rising and going to bed (grey lines) and the deviations of the transience times from these diary times. The individual values of these deviations are averaged and displayed on the plot as the difference with the corresponding average diary time rather than with zero. Vector lengths are inversely related to the dispersion of the differences (see Appendix of Chapter 6). HR, heart rate; MAP, mean arterial pressure; *, $p<0.05$ for difference with zero.*

Discussion

The square wave fit was applied for the modeling of circadian variation of heart rate and blood pressure profiles that were obtained by non-invasive measurement in outpatients. The diurnal variation was well modeled, as was judged by visual inspection. In a minority of the profiles, the modeling was improved by restriction of the allowed lengths of the low modeled period. In a previous study employing continuous intra-arterial blood pressure

recordings in hospitalized subjects[1], such a restriction was not necessary. Two reasons can be mentioned to explain this difference. First, the subjects in the present study continued their daily activities, and traveled to and from the hospital during the registration. In the subjects in the present study, blood pressure during the day was therefore probably less constant than in the subjects in the previous study. Indeed, in six of the blood pressure profiles recorded in the present study there was an interval of several hours during the day that was characterized by relatively high pressure. A second reason is the smaller number of measurements in the present study, which was on average 57, whereas the profiles in the previous study consisted of 72 data points. Moreover, each data point in the previous study was calculated by averageing more than 1000 measurements, which considerably reduces the inter-sample variation and therefore improves the quality of the fit. These facts also explain that the value 0.81±0.11 for cc_{max} of the square wave model of mean arterial pressure which we reported in the previous study, is larger than the value 0.71±0.12 which we found for the unrestricted square wave model in the present study.

In comparison with the previous study, we found a lower value of the square wave

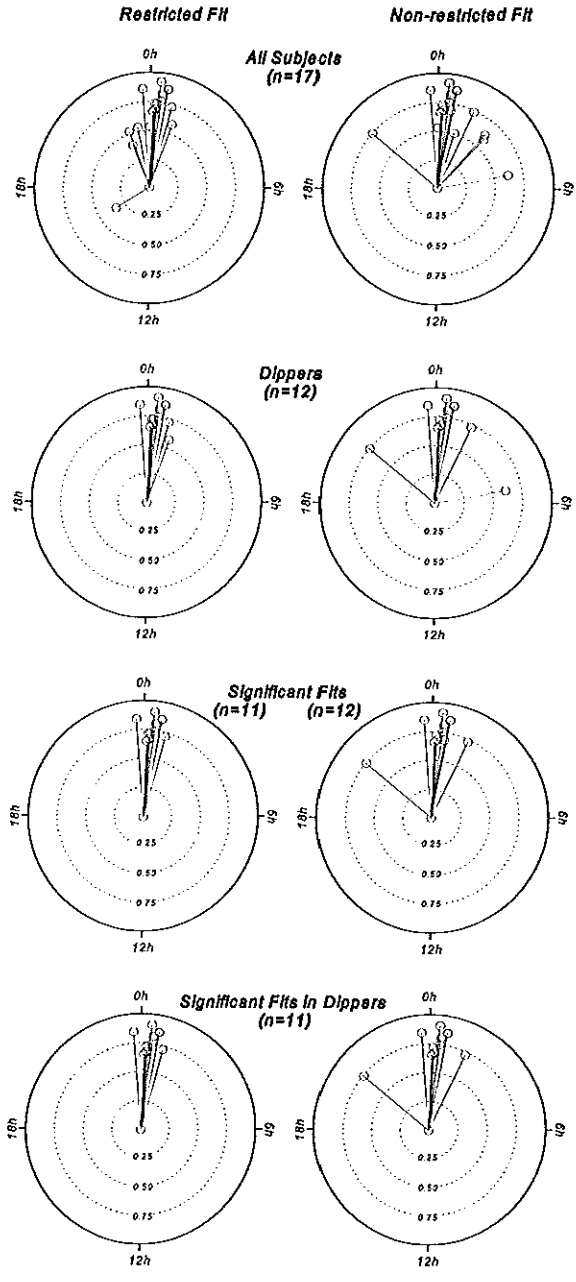

Figure 7. Circular plots show deviations of t_{up} from diary times of rise and cross correlation coefficients in mean arterial pressure profiles. The deviation of t_{up} from diary times is represented by the angle with the vertical axis and the cross correlation coefficient by the distance to the centre of the unit circle.

amplitude (15.7±7.2 mmHg vs 22.8±8.1 mmHg). It is well known that the nocturnal decrease of blood pressure is proportional to average 24-hour blood pressure[9]. Probably the lower average mean arterial pressure in the subjects in the present study (96±8.4 mmHg vs 112±13.1 mmHg in the previous study) can partially explain the lower value of the amplitude, although we were not able to demonstrate a relation between the square wave amplitude and the average 24-hour blood pressure. An alternative explanation may be that the sleep in the subjects in the present study was disturbed by the repeated cuff inflations of blood pressure measurement. Although Schwan and Eriksson[10] reported that non-invasive blood pressure measurement does not change the blood pressure during sleep, their study did not assess the effect of blood pressure measurement on baseline nocturnal blood pressure, but instead measured the short term reaction of blood pressure on nocturnal cuff inflation. Therefore, it cannot be excluded that during non-invasive blood pressure measurement baseline nocturnal blood pressure is elevated. The use of diary times for the calculation of diurnal blood pressure variation is cumbersome. Most often, investigators rely on the use of fixed clock times for the calculation of daytime and nighttime blood pressure[11]. The assumption of a fixed time period for the night rest in a group of unrestricted subjects, however, is probably not realistic. In the present study, for example, there was a wide range of times of awakening and going to bed, and the period during which all subjects were in bed had a length of 3 hours only. The transience times estimated by the square wave fit from mean arterial pressure were correlated to the diary times of rising and going to bed, and the average differences are small and insignificant. The square wave fit can therefore be used to estimate the daytime and nighttime blood pressure directly from the recorded profiles. The square wave fit therefore probably assesses the diurnal blood pressure variation better than the use of fixed clock times, in particular in unsynchronized subjects. This hypothesis is supported by a recent study by Fagard et al[8] of 24-hour blood pressure profiles recorded in healthy young men during a weekend when they went to bed at variable and often unusual times. The diurnal variation was modeled by means of two fixed clock time methods, a cusum technique, and the square wave fit. The nocturnal change of blood pressure calculated from the diary period of bed rest was best estimated by the square wave fit, both with respect to accuracy and precision. When the analysis was restricted to a subset with a usual sleeping pattern, the square wave fit remained the best estimator, with exception of a fixed clock time model that excluded the transience periods between the day and the night from the analysis, and that was slightly more accurate.

Although in all but one profiles of mean arterial pressure there was an evident diurnal rhythm of blood pressure, six of the 17 restricted square wave fits did not reach statistical significance. This may be explained by a small nocturnal decrease of blood pressure in these profiles, as was demonstrated in Figure 4 and in the Monte Carlo experiments in Chapter 6. Also, the considerable blood pressure variation during the day that was observed in some profiles partially obscures the square wave pattern of the profile. Another important reason is that the number of observations in the profiles is rather small for the estimation of the four parameters of the square wave model. The limited number of measurements has a detrimental effect on the statistical power of the fit, and therefore on the fraction of signifcant fits, as was demonstrated in our Monte Carlo experiments. In the present registrations consisting of on average 57 samples, fits were significant in profiles with a nocturnal decrease in blood pressure larger than approximately 10 mmHg.

Increasing the number of measurements in the 24-hour blood pressure profile will not only increase the accuracy and precision of the square wave fit parameter estimation, but will also increase the sensitivity of the fit. This results in significant fits in profiles having a nocturnal blood pressure decrease that is smaller than 10 mmHg, and in a larger fraction of significant fits.
We conclude that the square wave fitting may be used to assess the diurnal variation of heart rate and blood pressure in non-invasive blood pressure measurements in outpatients. It is advisable to restrict the lenght of the low period to a range from 5 to 13 hours.

References

1. Idema RN, Gelsema ES, Wenting GJ, Grashuis JL, van den Meiracker AH, Brouwer ML, Man in 't Veld AJ: A new method for diurnal blood pressure profiling - square wave fit compared with conventional methods. Hypertension 1992:19:595-605
2. Young MA, Rowlands DB, Stallard TH, Watson RDS, Littler WA: Effect of environment on blood pressure: home versus hospital. Br Med J 1983;286:1235-1236
3. Parati G, Bosi S, Castellano M et al: Guidelines for 24-hour non-invasive ambulatory blood pressure monitoring. Report from a working group of the Italian Societh of Hypertension. High Blood Press 1995,4:168-174
4. Millar-Craig MW, Hawes D, Whittington J: New system for recording ambulatory blood pressure in man. Med Biol Eng Comput 1978;16:727-731.
5. Davis JC: Statistics and data analysis in geology, 2nd edition. New York: John Wiley & Sons Inc., 1986
6. Shapiro SS, Wilk MB: An analysis of variance test for normality (complete samples). Biometrika 1965;52:591-611.
7. Lehmann EL: Nonparametrics: Statistical methods based on ranks. San Francisco: Holden-Day, Inc., 1975
8. Fagard R, Brguljan J, Thijs L, Staessen J. Prediction of the actual awake and asleep blood pressure by various methods of 24 h pressure analysis. J Hypertens 1996;14: 557-563
9. Pickering TG: Diurnal rhythms and other sources of blood pressure variability in normal and hypertensive subjects. In: Laragh JH, Brenner BM, eds. Hypertension: pathophysiology, diagnosis, and management. Raven Press, Ltd., New York, 1990. p. 1399
10. Schwan Å, Eriksson G: Effect on sleep -but not on blood pressure- of nocturnal non-invasive blood pressure monitoring. J Hypertens 1992;10:189-194
11. Van Ittersum FJ, Yzerman RG, Stehouwer CDA, Donker AJM: Analysis of 24-hour ambulatory blood pressure monitoring: what time period to assess blood pressure during waking and sleeping? J Hypertens 1995;13:1053-1058

Chapter 8

Square Wave Modeling of Circadian Variation of Heart Rate and Blood Pressure in Renal Transplant Recipients on Cyclosporine vs Azathioprine

R.N. Idema, M.A. van den Dorpel, A.H. van den Meiracker, E.S. Gelsema, A.J. Man in 't Veld

Based on data of *Van den Dorpel MA, van den Meiracker AH, Lameris ThW, Boomsma F, Levi M, Man in 't Veld AJ, Weimar W, Schalekamp MADH: Cyclosporine A impairs the nocturnal blood pressure fall in renal transplant recipients. Hypertension 1996 (in press)*

Introduction

In the previous chapters, we have introduced the square wave fit for the modeling of circadian blood pressure variation. In Chapter 4, the model was evaluated for blood pressure profiles recorded intra-arterially in hypertensive but otherwise healthy subjects having a normal circadian variation of blood pressure. In Chapter 6 the dependency of the square wave fit on the amplitude of the circadian variation of blood pressure, the measurement frequency, and the ratio of the lengths of the low and high pressure periods was investigated. It was concluded that the square wave fit may be used to characterize the circadian variation of blood pressure recorded by 60 cuff measurements in 24 hour in subjects having a normal circadian variation of blood pressure. The fraction of significant fits may be used to assess whether this condition is fullfilled, i.e., whether the circadian variation of blood pressure in the study group is sufficient to apply the square wave fit. In Chapter 7, the circadian blood pressure variation was modeled in profiles consisting of cuff measurements obtained in outpatients having a normal circadian blood pressure variation. In some of these subjects, the quality of the fit improved if the length of the low period was restricted to a range of 5 to 13 hours. This appeared to be the case in subjects in whom the unrestricted square wave fit was determined by a period of several hours of high pressure, which was probably related to the physical activity during the day.
In the present study we illustrate the effect of a small amplitude of the circadian blood pressure variation on the estimation of the square wave fit parameters. For this purpose, the square wave fit is applied to registrations of renal transplant recipients, who may be expected to have an attenuated circadian variation of blood pressure[1]. The fit was applied twice in the same subjects under two different immunosuppressive regimens, i.e., prednisone and azathioprine vs. prednisone and cyclosporine A. In a previous analysis of these data[2], it was concluded that cyclosporine in comparison with azathioprine immunosuppression attenuates the circadian variation of blood pressure. The two different immunosuppressive regimens thus allowed us to assess the circadian blood pressure variation in identical subjects having two different blood pressure rhythms. In this population we measured the fraction of significant fits, the validity of the model parameters, and the effect of restricting the square wave fit.

Subjects and Methods

Subjects

The present study of square wave fitting employs blood pressure registrations recorded for a study of the effect of cyclosporine on hemodynamic, renal, and biochemical parameters[2]. Patients for this study were recruited among renal transplant recipients who were enrolled in a prospective randomized clinical trial, which was designated to evaluate the effects of two different immunosuppresive regimens, i.e., prednisone combined with cyclosporine or prednisone combined with azathioprine, on long-term graft function and incidence of rejection periods. For this trial cyclosporine-treated renal transplant recipients

who were 6 months or longer after transplantation, were randomly allocated to either continuation of cyclosporine treatment, or conversion from cyclosporine to azathioprine-based immunosuppresion. For the present study renal transplant recipients without pre-existent hypertension, a clinic blood pressure of 150/95 mmHg or higher during antihypertensive treatment, and who were allocated to conversion from cyclosporine to azathioprine, were selected. Patients with diabetes mellitus, previous graft rejection or signs of autonomic neuropathy were excluded. The first 18 consecutive patients fulfilling these criteria and willing to give written informed consent were studied. About 50% of the patients who were converted from cyclosporine to azathioprine did not meet all criteria, mainly because of pre-existent hypertension which could not be attributed to the use of cyclosporine.

Blood Pressure Registrations

The first 24-hour blood pressure profiles were recorded while patients were on cyclosporine and the second measurement was done 16 weeks later when patients were on azathioprine therapy. During both studies the patients used the same dose of prednisone. All antihypertensive medication (betablockers in 13 patients and calciumchannel blockers in eight patients) was discontinued three days prior to the studies. Ambulatory blood pressure registrations were recorded by means of a Spacelabs ambulatory blood pressure measurement device (Spacelabs monitor model 90207,

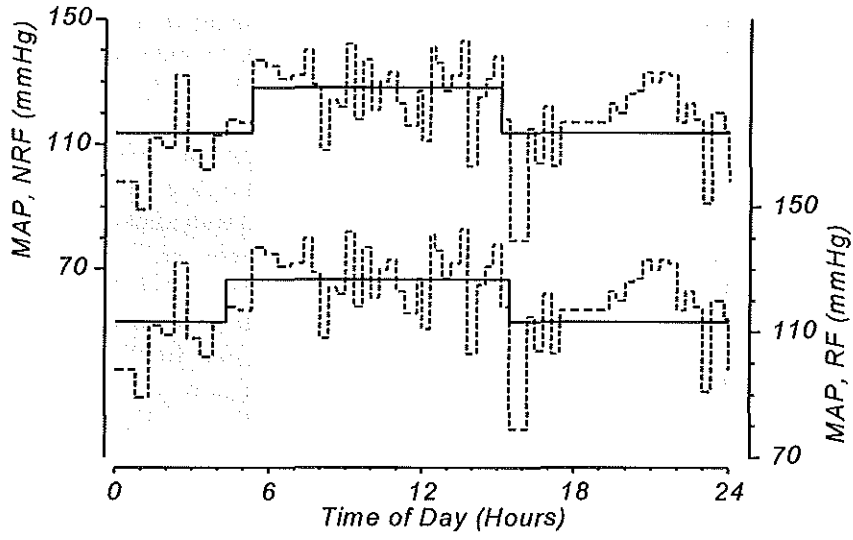

Figure 1. Profile of mean arterial pressure recorded in subject 2 using azathioprine immunosuppression. At 15:28 hour, mean arterial pressure was measured as 79 mmHg, whereas the measurement 20 minutes later failed. This combination induces a dip in the profile that is approximately 30 mmHg deep and has a width of 40 minutes. The square wave fit accomodates this prominent dip by modeling it in the low period, which therefore cannot start later than 15:28 hour. With restricted fitting, the length of the low period cannot exceed 13 hours, and therefore the end of the low period t_{up} cannot be later than 4:28 hour (lower profile), although the blood pressure rise to daytime level occurs at 5:19 hour. For this profile, the restricted square wave fit was classified as being less in accordance with the circadian pattern of blood pressure than the value of t_{up} obtained by non-restricted fitting (upper profile).

Spacelabs Inc. Redmond, WA, USA). Blood pressure was measured in the non-dominant arm. In patients who had undergone hemodialysis access surgery in the non-dominant arm, the dominant arm was used. From 7:00 hour to 22:00 hour blood pressure was measured at 20 minute intervals and from 22:00 to 7:00 hour at 30 minute intervals (63 measurements). Patients were advised to continue their activities during the recording period, but to be inactive and hang their arm by their side during each measurement. Patients kept a diary in which they noted the time of meals, their physical activities and the time they went to bed and woke up. Subjects with an interval of two hours or longer without any measurement were excluded from the study.

Analysis of Heart Rate and Blood Pressure Profiles

Outlier analysis was performed according to the method described in Chapter 7, which excludes measurements in which the value of either systolic pressure, diastolic pressure, mean arterial pressure, or heart rate, is outside the range mean±2.5*SD of the corresponding data distribution. In accordance with the previous analysis by van den Dorpel et al[2], the night-day difference was calculated from hourly averages, and the night was defined as the period in which all subjects were asleep, and the day as the period during which all subjects were awake (see Results). In addition, the circadian variation of heart rate and blood pressure was modeled by restricted and non-restricted square wave fitting as described in Chapter 7. The validity of square wave modeling of the material was assessed by calculating the percentage of fits that were significant, as described in Chapter 6. The restricted square wave fits were compared to the non-restricted fits by means of visual inspection, and the changes induced by restricting the square wave fit were classified as an improvement, a deterioration, or as an indifferent change (see Chapter 7).

Statistics

Clock time statistics were calculated by means of techniques for the analysis of directional data[3], using paired differences to assess the differences between groups, as has been described in the Appendix of Chapter 6. In addition, classical mean and standard deviations were calculated as an illustration of the difference between the two techniques. For the calculation of standard deviations the clock times were expressed as the difference with the average clocktime on a scale from -12:00 hour to 12:00 hour.

The numbers of significant fits were compared by a Fisher exact test[4]. For other parameters, normal distribution was tested by the Shapiro Wilk statistic[5]. Significance of difference from zero was assessed using paired or unpaired t-tests for normally distributed parameters and by a signed rank[6] test otherwise. For the assessment of statistical significance, a 0.05 significance level was chosen. All statistical computations were perfomed with SAS statistical software (SAS V6.04 for Personal Computers, SAS Institute Inc., Cary, North Carolina, USA).

Results

Subjects, Diaries and Registrations

In one of the subjects, a period of more than two hours was missing from one of the two registrations, and this subject was excluded from the analysis. After this exclusion, eleven male and six female kidney transplant recipients, aged 39±13 years, remained in the study. The time between transplantation and the first measurement ranged from 6 to 89 months (mean, 24 months). At the time of the first study the daily cyclosporine dose was 5.4±1.4 mg/kg, and at the time of the second study the azathioprine dose was 1.8±1.4 mg/kg. During both studies the dose of prednisone (mean, 10.4 mg; range, 7.5 to 12.5 mg) was identical. All subjects were awake from 9:00 to 22:00 hour, and all subjects were asleep from 1:00 hour to 5:30 hour.

After removal of outlying values (2% in the first and 3% in the second registration), 93±4% of all reading attempts of the first and 92±8% of the second recording remained in the profiles.

Restricted vs Non-restricted Square Wave Fitting

In approximately half of the profiles of heart rate, and in most of the profiles of mean arterial pressure recorded under azathioprine medication, the diurnal variation was modeled satisfactorily by non-restricted square wave fitting, as was judged by visual inspection. Restriction of the length of the low period of the square wave fit, however, gave a considerable improvement of the modeling of the diurnal variation (Table 1). An improvement occurred in profiles in which the non-restricted square wave fit was determined by a period of high pressure or heart rate during the day, or by a few outlying values. Most of these changes occurred in the profiles of heart rate. Indifferent changes, i.e., changes that were neither an improvement nor a deterioration, occurred in profiles in which no apparent diurnal rhythm was present. This was the case in most profiles of mean arterial pressure recorded during azathioprine immunosuppression. In one profile of mean arterial pressure (Figure 1), the modeling of diurnal variation was deterioriated by restricting the square wave length.

Table 1. Changes of Adequacy of Description of the Diurnal Variation of Blood Pressure and Heart Rate by Restricted vs Non-restricted Square Wave Fitting

	No Change	Indifferent Change	Improvement	Deterioration	Total
Heart rate (cyclo, aza)	17 (8, 9)	3 (2, 1)	14 (7, 7)	0	34 (17,17)
Mean arterial pressure (cyclo, aza)	17 (7, 10)	10 (7, 3)	6 (3, 3)	1 (0, 1)	34 (17, 17)

Changes are classified by visual comparison of restricted to non-restricted square wave fits. Values between brackets represent changes for cyclosporine (cyclo) and azathioprine (aza) separately.

Parameters of the 24-Hour Profiles of Heart Rate and Mean Arterial Pressure

The parameters calculated from the 24-hour profiles of heart rate and mean arterial pressure are summarized in Table 2. Compared to azathioprine immunosuppression, the average 24-hour blood pressure appears to be higher during cyclosporine medication (118 vs 107 mmHg, p=0.0001) whereas the nocturnal decrease in blood pressure calculated from fixed time periods is smaller (4.1 vs 11 mmHg, p=0.01). The diurnal variation of mean arterial pressure as assessed by the standard deviation over 24 hours, by the amplitude of the square wave fit, or by cc_{max}, does not differ between the immunosuppressive regimens. Similar to the observations in Chapter 7, the values of the amplitude and cc_{max} are larger, whereas t_{up} tends to be later and t_{down} tends to be earlier in

Table 2. Statistics of Diurnal Variation of Heart Rate and Mean Arterial Pressure in 17 Renal Transplant Recipients during Azathioprine and Cyclosporine Immunosuppression

	Azathioprine		Cyclosporine		Significance of Difference Between Medication (p)	
	HR	MAP	HR	MAP	HR	MAP
24-Hour average	86±11 bpm	107±14 mmHg	87±15 bpm	118±15 mmHg	ns	0.0001
Nocturnal change	-24±11 bpm*	-11±7.0 mmHg*	-22±11 bpm*	-4.1±10 mmHg	ns	0.01
Diurnal SD	16±4.3 bpm	10±1.9 mmHg	14±4.5 bpm	9.7±2.7 mmHg	ns	ns
Amplitude						
Restricted fit	23±7.8 bpm	14±4.3 mmHg	22±7.4 bpm	14±6.0 mmHg	ns	ns
Non-restricted fit	28±10 bpm	17±5.6 mmHg	28±13 bpm	19±7.2 mmHg	ns	ns
cc_{max}						
Restricted fit	0.67±0.13	0.56±0.13	0.69±0.10	0.53±0.14	ns	ns
Non-restricted fit	0.72±0.11	0.62±0.11	0.72±0.08	0.58±0.10	ns	ns
No. of significant fits						
Restricted fit	13	4	13	4	ns	ns
Non-restricted fit	14	7	14	5	ns	ns
t_{up} (h:m) [MDVL]						
Restricted fit	8:17 [0.95]†	7:58 [0.74]†	7:47 [0.91]†	9:18 [0.23]	ns	-
Non-restricted fit	8:46 [0.92]†	7:58 [0.74]†	8:55 [0.89]†	12:23 [0.18]	ns	-
t_{down} (h:m) [MDVL]						
Restricted fit	21:19 [0.88]†	22:14 [0.67]†	21:32 [0.86]†	22:10 [0.33]	ns	-
Non-restricted fit	18:38 [0.59]†	20:04 [0.33]	18:33 [0.54]†	20:59 [0.27]	ns	-

Values denote mean±SD, or, for clock times, the mean directional vector time and, between square brackets, mean directional vector length (MDVL). The nocturnal change difference is calculated as the difference between 1:00-6:00 hour, and 9:00h-22:00 hour. Diurnal standard deviation is calculated over 1 hour averages. *, p<0.0001; †, p<0.05; ns, not significant; -, not calculated because of non-significant values in at least one group.

non-restricted fits as compared to restricted fits.
The transience times will be discussed in detail in comparison with the diary times (see below). However, at this point we indicate that the average values of t_{up} and t_{down} are significant in the profiles of heart rate recorded under both immunnosuppressive regimens, in other words, they appear not be distributed randomly over the 24 hours. The average transience times modeled in mean arterial pressure are significant only in the profiles recorded under azathioprine medication. The scatter in the transience times calculated from heart rate and mean arterial pressure is smaller when these are modeled by restricted fitting. The scatter in t_{down} modeled by non-restricted square wave fitting in the profiles of mean arterial pressure recorded during azathioprine medication is so large that the average value of t_{down} does not reach statistical significance.

Comparison of Transience Time Values to Diary Time Values
In Figure 2, the individual values of t_{up} and t_{down} are compared to times of rising from and going to bed as recorded in the diaries. Statistics of these comparisons are summarized in Table 3 and in Figure 3. The times of rising and going to bed as recorded in the diaries differ only slightly and non-significantly between the two immunosuppresive regimens. For heart rate, the transience times calculated by square wave fitting differ from the times recorded in the diaries. On average, t_{up} is later than the time of rising, and t_{down} is earlier than the time of going to bed, in other words, the period of low heart rate is longer than the night rest. This pattern is independent of medication and is more pronounced if transience times are calculated by non-restricted fitting. In addition, the deviations of t_{up} and t_{down} from the corresponding diary times tend to be larger when the transience times are modeled by non-restricted instead of restricted square wave fitting. The average deviation of t_{down} from the time of going to bed and the scatter of this deviation are much larger than the deviation of t_{up} from the time of rise and the corresponding variation.
Most observations on the transience times calculated from the heart rate profiles are also true for the transience times calculated from the profiles of mean arterial pressure recorded during azathioprine medication. However, in these profiles the average value of t_{up} is not later than the time of rising. The period of low modeled blood pressure tends to be longer than the period of bed rest, but this is caused only by the values of t_{down} that are significantly earlier than the times of going to bed.
The values of t_{up} modeled in profiles of mean arterial pressure, when compared to the diary times (see upper right plot in Figure 2) suggest that some subjects in the cyclosporine group still have the normal increase of blood pressure at morning rise. These are the subjects depicted near the line of identity. In other subjects, however, the modeled increase of blood pressure occurs approximately 12 hours later, in the first part of the evening. Apparently the blood pressure profiles of these subjects are in anti-phase with the normal blood pressure profiles. For the whole group of mean arterial pressure profiles recorded under cyclosporine immunosuppression, the differences between the diary times and the corresponding transience times cannot be discerned from a random distribution.

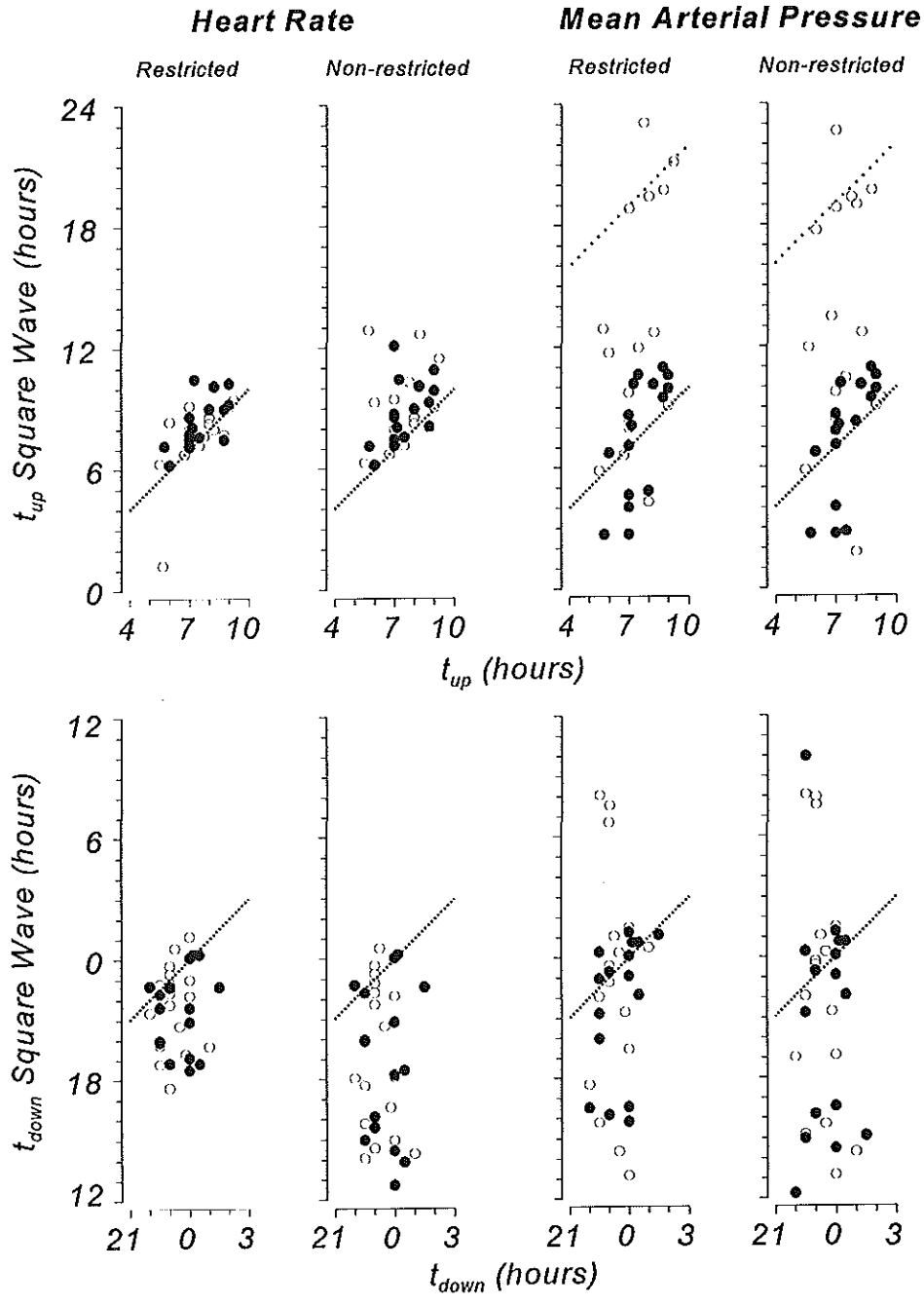

Figure 2. Comparison of values for t_{up} (upper plots) and t_{down} (lower plots) calculated in profiles of heart rate (left) and mean arterial pressure (right) by means of restricted (columns 1 and 3) and non-restricted (columns 2 and 4) square wave fitting. ●, values under azathioprine medication; ○, values under cyclosporine; "····", line of identity; "· · · ·", line of anti-phase with identity.

Table 3. Comparison of Square Wave Transience Times t_{up} and t_{down} to Diary Times of Rising and Going to Bed, respectively

	Azathioprine (●)		Cyclosporine (○)	
	HR	MAP	HR	MAP
Time of rising				
MDVT (h:m) [MDVL]	7:31 [0.97]*	7:31 [0.97]*	7:24 [0.96]*	7:24 [0.96]*
mean±SD (h:m)	7:31±1:00	7:31±1:00	7:24±1:07	7:24±1:07
t_{up}, restricted fit				
Δ MDVT (h:m) [MDVL]	0:48 [0.97]*†	0:10 [0.82]*	0:16 [0.93]*	4:08 [0.29]
Δ mean±SD (h:m)	0:48±1:00 ‡	0:04±2:25	0:12±1:30	5:19±5:34 ‡
t_{up}, non-restricted fit				
Δ MDVT (h:m) [MDVL]	1:17 [0.94]*†	0:09 [0.83]*	1:30 [0.88]*†	6:02 [0.23]
Δ mean±SD (h:m)	1:19±1:22 ‡	0:00±2:23	1:38±2:04 §	5:45±5:58 ‡
Time of going to bed				
MDVT (h:m) [MDVL]	23:32 [0.97]*	23:32 [0.97]*	23:15 [0.98]*	23:15 [0.98]*
mean±SD (h:m)	23:32±0:59	23:32±0:59	23:15±0:45	23:15±0:45
t_{down}, restricted fit				
Δ MDVT (h:m) [MDVL]	-2:07 [0.86]*†	-1:31 [0.70]*†	-1:34 [0.85]*†	-1:04 [0.30]
Δ mean±SD (h:m)	-2:08±2:07 ‡	-1:53±3:16	-1:37±2:15 †	-0:33±5:52
t_{down}, non-restricted fit				
Δ MDVT (h:m) [MDVL]	-4:33 [0.57]*†	-3:06 [0.37]	-4:17 [0.58]*†	-1:52 [0.20]
Δ mean±SD (h:m)	-4:41±3:57 §	-2:30±5:45	-4:18±3:51 §	-0.45±6:05

Table values indicate mean directional vector times (MDVT) and -lenghts (MDVL) of 17 subjects. Mean±SD are included in the table as an illustration of the difference between statistical techniques and do not represent study results. Δ, difference of modeled transience times with diary times; *, $p<0.05$ for non-uniform distribution of time values; † $p<0.05$, ‡ $p<0.01$, § $p<0.001$ for difference with zero.

Discussion

Twenty-four-hour Blood Pressure

In comparison to a previous analysis of these data[2], one subject in whom a period longer than two hours was missing during the morning period of one of the registrations was excluded. This did not alter the study results, i.e., an increase of 24 hour mean arterial pressure and an attenuation of the nocturnal decrease in blood pressure under cyclosporine in comparison to azathioprine immunosuppression. In the previous study[2] it was demonstrated that the differences between the immunosuppressive regimens were associated with an increase in renal vascular resistance, a decrease of renal blood flow and glomerular filtration rate, and higher levels of thromboxane B_2, prostaglandin E_2, and atrial natriuretic peptide during the use of cyclosporine.

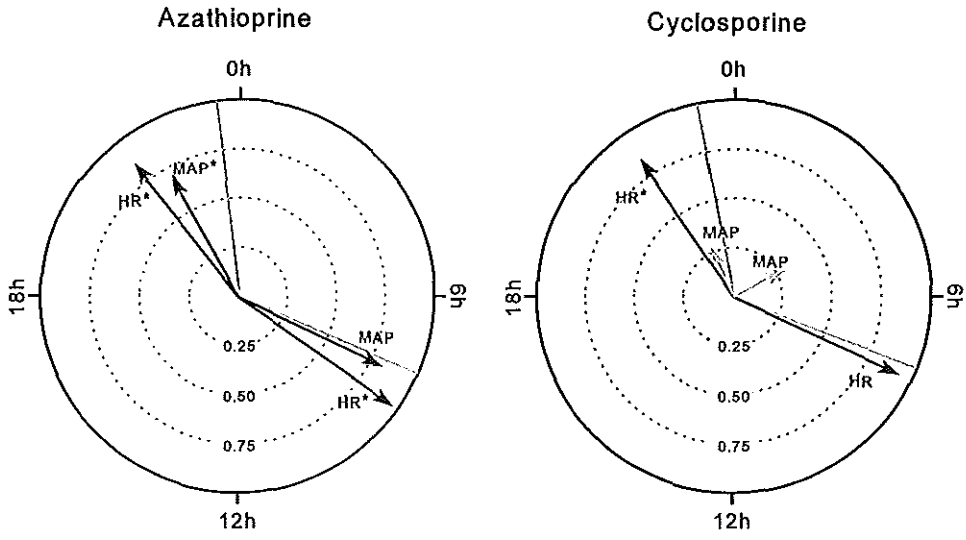

*Figure 3. Circular plots show average diary times of rising and going to bed (grey lines) and the deviations of the transience times obtained by restricted square wave fitting from these diary times. The individual deviations are averaged and displayed on the plot as the difference with the corresponding average diary time rather than with zero. Vector lengths are inversily related to dispersion (see Appendix of Chapter 6). Insignificant vectors are displayed in grey. HR, heart rate; MAP, mean arterial pressure; *, $p<0.05$ for difference with zero.*

Restricted vs Non-restricted Square Wave Fit

In accordance with the findings in Chapter 7, the modeling of diurnal variation of mean arterial pressure and, in particular, heart rate by the square wave fit was improved by restricting the length of the low period to a range of 5 to 13 hours. The improvement occurred in profiles in which the unrestricted fit was determined by outlying values or by a period of high heart rate or blood pressure during the day. Since restricted fitting appears to best model the circadian variation, we will discuss the results of the restricted fit only.

Validity of Square Wave Parameters

The validity of the application of the square wave fit to the present registrations was assessed by calculating the number of significant fits. Under the assumption that the circadian variation is sufficiently large to be modeled by the square wave fit (see Chapter 6), the expected percentage of significant fits is at least 40%. The number of significant square wave fits in the 17 profiles of heart rate in the present group was 14 (82%) during cyclosporine and 13 (76%) during azathioprine medication. These values are approximately identical to the values obtained for the heart rate profiles of the normotensive subjects described in Chapter 7 (11 out of 17 registrations, 65%), and suggest that the diurnal variation is reliably estimated by the square wave fit.

The number of significant fits in the profiles of mean arterial pressure recorded in the present renal transplant recipients during both cyclosporine and azathioprine medication was considerably smaller than in the normotensive subjects described in Chapter 7 (24% vs 65%). Therefore the square wave parameters of mean arterial pressure in the renal

transplant recipients under both immunosuppressive regimens must be considered unreliable. This explains that we did not find a difference in the magnitude of the circadian variation of mean arterial pressure between both groups when this was modeled by the square wave amplitude or cc_{max}, whereas the diurnal variation of blood pressure assessed by a conventional method, i.e., calculated over fixed time periods, was smaller during cyclosporine than during azathioprine medication. In Chapter 6 we found that the bias of the square wave fit amplitude estimation increases as the amplitude of the diurnal variation decreases.
This also explains the similar values of amplitude of the circadian variation of mean arterial pressure in the normotensive subjects in Chapter 7, and the present renal transplant recipients. When the magnitude of the circadian variation was modeled as cc_{max} , it appeared to be significantly smaller in the renal transplant recipients under both immuno-suppressive regimens than in the normotensive subjects (two-tailed t-test, p=0.04; data not shown).
The precision and accuracy of the square wave parameter estimation can be increased by increasing the sampling frequency, providing at least that the amplitude of the diurnal variation is larger than the between-measurement standard deviation (see Chapter 6). In the present material, however, the amplitude of the diurnal variation as calculated from fixed time periods (4.1 mmHg) is much smaller than the between-measurement standard deviation, which is approximately 8 mmHg (data not shown). Therefore, the amplitude of the diurnal variation cannot be calculated in the present recordings by means of the square wave fit.

Transience Times
The differences of the transience times with the diary times have an identical pattern in the renal transplant recipients as in the normotensive subjects, i.e., a tendency to later values for t_{up} and earlier values for t_{down} althought the differences with the diary times and the scatter therein appears to be larger in the renal transplant recipients. Moreover, during cyclosporine medication, the differences of the transience times with the diary times in profiles of mean arterial pressure appear to be randomy distributed. A closer look at the distribution of values for t_{up} calculated from mean arterial pressure profiles recorded under cyclosporine medication suggests that some subjects may have a normal rhythm whereas others appear to have an inverted rhythm. Such a inversion of phase of the diurnal blood pressure rhythm has been observed by other authors in subjects receiving high doses of glucocorticoids[7,8].

Clock Time Statistics
The present material illustrates the necessity for appropriate statistical handling of clock time values. For example, the average difference between the values of t_{up} calculated by restricted square wave fitting in profiles of mean arterial pressure recorded under cyclosporine medication and the corresponding diary times appears to be 5:19±5:34 hour when calculated as the mean±SD which is highly significant by the signed rank test. The appropriate statistic, i.e., Rayleigh's test for the presence of a preferred trend, reveals that in fact a significant deviation from diary time cannot be demonstrated in the present material. The different results of the two techniques can be understood by observing the corresponding scatter plot (Figure 2). In the signed rank test the values of the observations

between 17:00h and 22:00h which are located under the line of anti-phase, are all 11 to 12 hours later than the diary time of rise. These observations will all increase the average value of the difference considerably. In addition, high positive rank numbers will be assigned to these observations, which causes the overestimation to become highly significant. However, if the circular aspect of these data is appreciated, it appears that these observations instead abolish the significance of the overestimation, since they point in the opposite direction of the time of rising. This is illustrated in the scatter plot by their presence near the line of anti-phase.

Conclusions

The present study confirms that restricting the square wave fit improves the modeling of circadian variation of heart rate and blood pressure, according to visual inspection of the profiles of heart rate and blood pressure, and according to the correspondence of square wave transience times and the period of bed rest as recorded in the subject diaries. The square wave fit cannot be used, however, as a quantitative measure of circadian blood pressure variation if the magnitude of the diurnal variation is too small, as is the case in the present group of renal transplant recipients. Whether the diurnal variation is sufficient for application of the square wave fit can be determined by the percentage of fits that is significant (see Chapter 6). If the diurnal variation is too small to obtain a quantitative measure, qualitative information on the diurnal variation may still be obtained from the phase of the square wave fit. The parameter t_{up} best represents the phase since it better corresponds to the period of bed rest than t_{down}. By means of this parameter we found a significant blood pressure rhythm during azathioprine but not during cyclosporine immunosuppression. For a quantitative measure of circadian variation, however, one must rely on the (unbiased) calculation of diurnal variation based on diary times or on one of the many fixed clock time calculation schemes[9].

References

1. Baumgart P, Walger P, Gemen S, von Eiff M, Radt H, Rahn KH: Blood pressure elevation during the night in chronic renal failure, hemodialysis and after renal transplantation. Nephron 1991;57:293-298
2. Van den Dorpel MA, van den Meiracker AH, Lameris ThW, Boomsma F, Levi M, Man in 't Veld AJ, Weimar W, Schalekamp MADH: Cyclosporine A impairs the nocturnal blood pressure fall in renal transplant recipients. Hypertension 1996 (in press)
3. Davis JC: Statistics and data analysis in geology, 2nd edition. New York: John Wiley & Sons, Inc., 1986
4. Diem K, Lentner C, eds. Wissenschaftliche Tabellen, 7th edition. Basle: J.R. Geigy A.G., Pharma, 1986, p. 117, p. 123
5. Shapiro SS, Wilk MB: An analysis of variance test for normality (complete samples). Biometrika 1965;52:591-611
6. Lehmann EL: Nonparametrics: Statistical methods based on ranks. San Francisco: Holden-Day, Inc., 1975
7. Van de Borne Ph, Leeman M, Primo G, Degaute JP: Reappearance of a normal circadian rhythm of blood pressure after cardiac transplantation. Am J Cardiol 1992;69:794-801
8. Imai Y, Abe K, Sasaki S et al: Exogenous glucocorticoid eliminates or reverses circadian blood pressure variations. J Hypertens 1989;11:113-120
9. van Ittersum FJ, IJzerman RG, Stehouwer CDA, Donker AJM: Analysis of twenty-four-hour-ambulatory blood pressure monitoring: what time period to assess blood pressures during waking and sleeping? J Hypertens 1995;13:1053-1058

Chapter 9

Decreased Circadian Blood Pressure Variation Up to Three Years After Heart Transplantation

R.N. Idema, A.H. van den Meiracker, A.H.M.M. Balk, E. Bos, M.A.D.H. Schalekamp, A.J. Man in 't Veld

Published in The American Journal of Cardiology 1994;73:1006-1009

Supported by a grant-in-aid for scientific research from the Netherlands Heart Foundation (NHS 86.070) and by the Cardiovascular Research Foundation Rotterdam.

Introduction

The normal circadian blood pressure variation has been reported to be attenuated or completely abolished in cardiac transplant recipients[1-3]. The reason for this attenuation is not clear, but it has been suggested to be related to the force-fed pump characteristics of the denervated heart or the use of glucocorticoids, or both[1,4]. A consequence of the attenuation or abolition of the nocturnal reduction in blood pressure is an increase in the 24-hour pressure load, which may be especially injurious in cardiac transplant recipients, because of the development of cyclosporine-induced hypertension in a large proportion of these subjects. Recently, evidence was provided that attenuation of the circadian blood pressure variation soon after cardiac transplantation disappears within one year and that a normal circadian rhythm of blood pressure is restored[5,6]. In the present study, we report the results of invasive 24-hour blood pressure recording in cardiac transplant recipients 11 to 36 months after transplantation. The findings indicate that more than one year after cardiac transplantation, an attenuation of the circadian BP variation remains in most subjects.

Table 1. Age, Sex, Time after Transplantation, and Dosages of Cyclosporine and Prednisone in 17 Cardiac Transplant Recipients

Subject	Age (yr)	Sex	Time after transplantation (months)	Cyclosporine (mg/kg)	Prednisone (mg/kg)
1	18	M	13	5.3	0.18
2	22	M	12	14.9	0.15
3	30	F	21	5.4	0.11
4	32	F	36	9.1	0.15
5	32	M	33	6.3	0.13
6	38	M	12	7.1	0.14
7	38	M	12	6.1	0.15
8	42	M	24	3.3	0.11
9	43	M	12	5.5	0.11
10	44	M	12	4.6	0.09
11	44	M	24	7.6	0.12
12	44	M	12	7.3	0.15
13	48	M	11	7.9	0.13
14	49	M	36	8.2	0.26
15	49	M	12	6.6	0.13
16	56	M	11	6.6	0.13
17	56	F	12	9.7	0.16
Mean	40		17	7.1	0.14

Subjects and Methods

Subjects

Invasive 24-hour ambulatory blood pressure recordings were obtained in 17 cardiac transplant recipients 17 months (range 11 to 36) after transplantation. Clinical characteristics of patients, the time intervals between transplantation and blood pressure recording, and the dosages of cyclosporine and prednisone are listed in Table 1. No patient received any antihypertensive agent at the time of the blood pressure recording. Invasive 24-hour ambulatory blood pressure recordings were obtained also in 17 subjects who were matched for age and average 24-hour mean arterial pressure. Recordings in subjects were obtained as part of the assessment of their hypertension. No subject used any medication for at least 3 weeks before the blood pressure recording. All subjects gave informed consent to participate in the study, which was approved by the Medical Ethical Committee of the University Hospital "Dijkzigt". All subjects were in the hospital at the time of the blood pressure recording to standardize environmental conditions. During the recordings, subjects were free to move within the hospital, but had their meals at fixed times, went to bed at 22:30 hour and were awakened at 7:00 hour.

Blood Pressure Recordings

Ambulatory blood pressure was measured according to previously described methods. Using the Seldinger technique, a 10 cm long, 1 mm diameter Teflon catheter was inserted into the brachial artery of the nondominant arm after local anesthesia with a 2% lidocaine solution. The catheter was connected to a miniature transducer-perfusion device which was fitted in front of the chest at the level of the heart. The transducer signal was recorded on magnetic tape with a portable tape recorder (Medilog Recorder II, Oxford Medical Instruments, Oxford, United Kingdom).

Data Analysis

The blood pressure signals were analysed off-line by a computer program that calculated systolic, diastolic and integrated mean arterial pressure, pulse pressure, and pulse interval of individual beats. The diurnal variations of blood pressure and heart rate were quantified as the differences between average day and night values. Transient periods between day and night were removed from the calculations by defining night as the period from 0:00 hour to 6:00 hour and day as that from 8:00 hour to 20:00 hour.

Values are presented as mean±SD. Student's t test was used for comparison. A p-value <0.05 was considered statistically significant.

Results

The mean age of the 17 control subjects (one woman and 16 men) was 42±11 years. Average 24-hour mean arterial pressure was 107±14 mmHg in cardiac transplant recipients and 107±13 mmHg in control subjects. The group mean profiles of 24-hour

ambulatory mean arterial pressure and heart rate are shown in Figure 1. In the control group, blood pressure markedly decreased during the night, whereas only a small, nocturnal reduction was observed in the transplant group (Table 2). In contrast to this attenuated day-night fluctuation, blood pressure in cardiac transplant recipients fluctuated more during the day than in control subjects. In the transplant group, three short-lasting but prominent reductions in blood pressure occurred at 8:00 hour, 12:00 hour, and 18:00 hour (i.e., soon after the meals). To quantify the effects of these decreases on daytime blood pressure, and hence on the difference in blood pressure between day and night, the beginning and end points of the decreases were identified visually, and an artificial blood pressure profile without the decreases was constructed by interpolation. As a result of this procedure, average daytime blood pressure increased by 2.2 mmHg. In regard to the blood pressure data of individual subjects, only one cardiac transplant recipient had a nocturnal reduction in blood pressure that was larger than the average nocturnal decrease in control subjects (Figure 2).

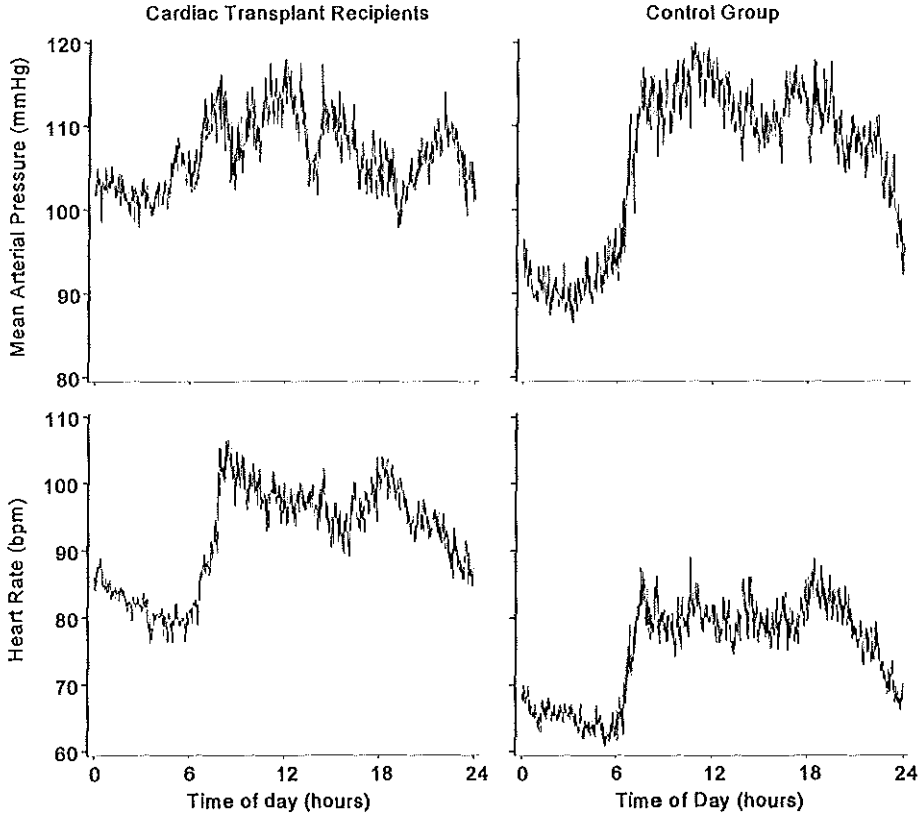

Figure 1. Profiles of invasive 24-hour ambulatory blood pressure recordings in 17 cardiac transplant recipients and 17 control subjects. Group means of 1-minute averages of mean arterial pressue and heart rate are depicted.

Table 2. Average Day and Night Values of Systolic, Diastolic and Mean Arterial Pressures, Pulse Pressure and Heart Rate, and the Difference between These Values in Cardiac Transplant Recipients and Control Subjects

	Cardiac transplant recipients			Control subjects		
	Day	Night	Night-Day Difference	Day	Night	Night-Day Difference
Systolic arterial pressure (mmHg)	142±17	132±18	-10±12*	154±18	124±17	-31±12‡§
Mean arterial pressure (mmHg)	108±13	103±15	-5±9†	114±15	93±13	-21±9‡§
Diastolic arterial pressure (mmHg)	90±11	86±12	-4±7†	91±12	74±10	-18±8‡§
Pulse pressure (mmHg)	51±10	46±9	-6±6*	63±11	50±10	-13±8*§
Heart rate (beats/min)	105±13	83±13	-16±7‡	82±11	65±7	-15±8‡

Values are mean±SD. *, $p<0.01$; †, $p<0.05$; ‡, $p<0.001$ for within-group differences.
§, $p<0.001$ for between-group differences.

Heart rate was significantly higher in cardiac transplant recipients than in control subjects, but the day-night difference in the two groups was of similar magnitude (Table 2).

Discussion

The principal finding of this study is the persistence of an abnormally low diurnal variation of blood pressure in most patients one to three years after cardiac transplantation. The findings confirm some previous studies using noninvasive ambulatory blood pressure recording techniques[1-3], but are at variance with those of van de Borne et[5]al and von Pölnitz et al[6]. Those investigators found a reappearance of the normal circadian variation of blood pressure late after cardiac transplantation. They both speculated that a reduction in the dose of glucocorticoids after chronic cardiac transplantation could explain their observation. This explanation remains questionable because the average daily dose of glucocorticoids in the present study was similar to those used in the two aforementioned studies.

With regard to medical treatment, the present subjects differed from those studied by van de Borne et al[5] and von Pölnitz et al[6], in two important aspects. First, the average daily dose of cyclosporine of 7.1 mg/kg of body weight in the present study was much higher than that of 4.2 mg/kg of body weight in those of the other two studies. The subjects needed this higher dose of cyclosporine, because they did not use the immunosuppressant azothioprine. Cyclosporine is associated with an increase in blood pressure. Although the mechanism responsible for this increase is not completely clear, cyclosporine has been reported to cause fluid retention[8] and an increase in sympathetic nerve activity[9]. If these effects of cyclosporine are dose-dependent, then the attenuation of the nocturnal blood pressure reduction observed in the present study may be related to the relatively high dose of cyclosporine, although we were unable to show a relation between the daily dose of

cyclosporine used in cardiac transplant recipients and the nocturnal reduction in blood pressure. Second, the present subjects, in contrast to most of those in the aforementioned two studies, did not use antihypertensive medication. The antihypertensive medication used by subjects in the aforementioned two groups predominantly consisted of of calcium antagonists, angiotensin-converting enzyme inhibitors, and diuretics. There is no evidence that these agents have important influences on the 24-hour blood pressure profile in normal hypertensive subjects, but their effect on diurnal blood pressure variation in heart transplant recipients is unknown. However, no relation between antihypertensive drug therapy and absence of a nocturnal reduction in blood pressure was found in cardiac transplant recipients studied by Reeves et al[1].

Although the present cardiac transplant recipients did not use antihypertensive medication, this does not mean that their blood pressure was not increased. Average daytime ambulatory blood pressure in cardiac transplant recipients was 142/90 mmHg. Using the same technique of blood pressure recording as in the present study and in a comparable environmental setting, Pomidossi et al[10] reported an average daytime ambulatory blood pressure in a group of normotensive and borderline hypertensive subjects of 120/64 mmHg (i.e., a considerably lower value than in cardiac transplant recipients).

A phenomenon that to the best of our knowledge has not been reported in cardiac transplant recipients was three prominent decreases in blood pressure during the day occurring soon after eating. Ingestion of food is associated with vasodilatation in the splanchnic circulation[11]. In healthy subjects, this vasodilatation is usually not accompanied by a reduction in blood pressure, because of an increase in cardiac output and reflex-vasoconstriction in other vascular beds [12,13]. Presumably owing to the absence of cardiac sympathetic innervation, the increase in cardiac output in response to postprandial vasodilatation is insufficient after heart transplantation and explains why ingestion of food in this condition is associated with a reduction in blood pressure. Although average daytime blood pressure was lowered by the postprandial reductions in blood pressure, this effect was insufficiently large to significantly influence the day-nigh difference in blood pressure. We estimated that without the postprandial reductions, the day-night difference in mean arterial pressure would increase from 5.3 to 7.5 mmHg, which is markedly lower than the average 20.5 mmHg day-night difference observed in the control group.

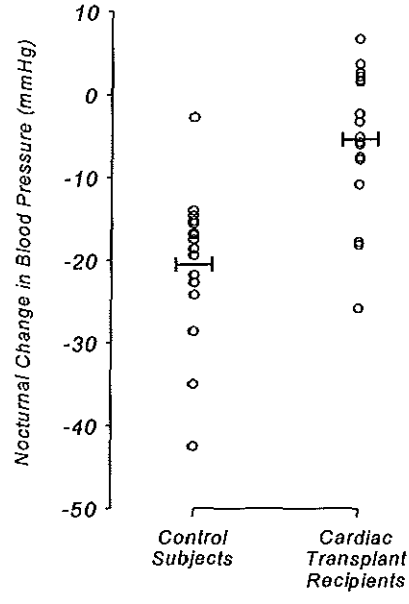

Figure 2. Individual changes in nocturnal mean arterial pressure compared with average daytime values in cardiac transplant recipients and control subjects.

Heart rate in cardiac transplant recipients is increased owing to the denervated state, particularly the absence of vagal tone. In

the present study, heart rate in cardiac transplant recipients was almost 20 beats/min higher than in control subjects. This higher heart rate was observed during both the day and night periods; therefore, the absolute day-night difference of heart rate in cardiac transplant recipients was similar to that in control subjects. The higher heart rate after heart transplantation explains why, notwithstanding similar values of 24-hour mean arterial pressure, pulse pressure was significantly lower in cardiac transplant recipients than in control subjects.

Thus, attenuation of the normal circadian variation of blood pressure may occur up to three years after heart transplantation. In addition, cardiac transplant recipients may have marked postprandial reductions in blood pressure, which because of their relatively short duration have only a small effect on the day-night difference of blood pressure.

References

1. Reeves RA, Shapiro AP, Thompson ME, Johnsen AM. Loss of nocturnal decline in blood pressure after cardiac transplantation. Circulation 1986;73:401-408
2. Wenting GJ, van den Meiracker AH, Simoons ML, Bos E, Ritsema van Eck HJ, Man in 't Veld AJ, Weimar W, Schalekamp MADH. Circadian variation of heart rate but not of blood pressure after cardiac transplantation. Transplant Proc 1987;19:2554-2555
3. Sehested J, Thomas F, Thorn M, Schifter S, Regitz V, Sheikh S, Oelkers W, Palm U, Meyer-Sabellek W, Hetzer R. Levels and diurnal variations of hormones of interest to the cardiovascular system in patients with heart transplants. Am J Cardiol 1992;69:397-402
4. Imai Y, Abe K, Sasaki S, Minami N, Munakata M, Nihei M, Sekino H, Yoshinaga K. Exogenous glucocorticoid eliminates or reverses circadian blood pressure variations. J Hypertens 1989;7:113-120
5. Van de Borne P, Leeman M, Primo G, Degaute JP. Reappearance of a normal circadian rhythm of blood pressure after cardiac transplantation. Am J Cardiol 1992;69:794-801
6. Von Pölnitz A, Bracht C, Kemkes B, Höfling B. Circadian pattern of blood pressure and heart rate in the longer term after heart transplantation. J Cardiovasc Pharmacol 1990;15:S86-S89
7. Millar-Craig MW, Haws D, Whittington J. New system for recording ambulatory pressure in man. Med Biol Eng Comput 1978;16:727-731
8. Bantle JP, Boudreau RJ, Ferris TF. Suppression of plasma renin activity by cyclosporine. Am J Med 1987;83:59-64
9. Scherrer U, Vissing SF, Morgan BJ, Rollins JA, Tindall RSA, Ring S, Hanson P, Mohanty PK, Victro RG. Cyclosporine-induced sympathetic activation and hypertension after heart transplantation. N Engl J Med 1990;323:693-699
10. Pomidossi G, Parati G, Casadei R, Villani A, Gropelli A, Mancia G. Twenty-four-hour ambulatory intra-arterial blood pressure in normotensive and borderline hypertensive subjects. J Hypertens 1988;6(suppl 4):S67-S69
11. Waaler BA, Ericksen M, Janbu T. The effect of a meal on cardiac output in man at rest and during moderate exercise. Acta Physiol Scand 1990;140:167-173
12. Fagan TC, Sawyer PR, Gourley L, Lee JT, Gaffney TE. Postprandial alterations in hemodynamics and blood pressure in normal subjects. Am J Cardiol 1986;58:636-641
13. Lipsitz LA, Ryan SM, Parker JA, Freeman R, Wei JY, Goldberger AL. Hemodynamic and autonomic nervous system responses to mixed meal ingestion in healthy young and old subjects and dysautonomic patients with postprandial hypotension. Circulation 1993;87:391-400

Chapter 10

Abnormal Diurnal Variation of Blood Pressure, Cardiac Output and Vascular Resistance in Cardiac Transplant Recipients

R.N. Idema, A.H. van den Meiracker, A.H.M.M. Balk, E. Bos, M.A.D.H. Schalekamp, A.J. Man in 't Veld

Published in Circulation 1994;90:2797-2803

Supported by a grant-in-aid for scientific research from the Netherlands Heart Foundation (NHS 86.070) and by the Cardiovascular Research Foundation Rotterdam

Summary

An attenuated or absent nocturnal decline in blood pressure has repeatedly been documented in cardiac transplant recipients. The present study was aimed to investigate the hemodynamic mechanism underlying this abnormality.
In 23 cardiac transplant recipients (11 to 36 months after transplantation) and in 23 control subjects, matched for age and 24-hour mean arterial pressure, invasive 24-hour ambulatory blood pressure was measured by means of the Oxford technique. Beat to beat relative values of stroke volume were determined by means of a pulse contour method and relative changes of cardiac output (stroke volume × heart rate) and total peripheral vascular resistance (blood pressure / cardiac output) over the 24-hour period were calculated. The nocturnal decline in blood pressure was 20±8% (mean±SD) in control subjects, but only 5±9% ($p<0.001$) in cardiac transplant recipients. In control subjects the nocturnal decline in blood pressure was associated with a nocturnal fall in cardiac output of 24±13%, whereas vascular resistance as compared to day-time value did not change. The small nocturnal decline in blood pressure in cardiac transplant recipients was associated with an attenuated nocturnal fall in cardiac output of 14±12% ($p<0.05$ vs controls). In addition, vascular resistance as compared to day-time value was increased by 9±9% ($p<0.05$) during the night. Both in cardiac transplant recipients and in control subjects the nocturnal changes in blood pressure correlated with the nocturnal changes in cardiac output but not with the nocturnal changes in total peripheral vascular resistance. This study confirms the attenuated nocturnal fall in blood pressure in cardiac transplant recipients. Hemodynamically this attenuated blood pressure decline is characterized by a reduced nocturnal fall in cardiac output and it is associated with a nocturnal increase in vascular resistance.

Introduction

The normal nocturnal decline in blood pressure is absent or attenuated after heart transplantation[1-6]. The mechanism underlying this hemodynamic abnormality is not known. It has been suggested that during the night cardiac output as compared to the resistance in the arteriolar vascular tree is relatively high[1,2]. This "inappropriately" high cardiac output might be explained by an increase in venous return during nighttime recumbency, for which, contrary to the normally innervated heart, no compensation occurs by means of a decrease in cardiac inotropy and/or chronotropy. A contributory factor to this nocturnal mismatch between cardiac output and arteriolar vascular resistance could be the impairment of the restraint of sympathetic outflow to the vascular tree due to the interruption of the ventricular-baroreceptor reflex after heart transplantation[7,8].
The use of immunosuppressive therapy may also be involved in the attenuation of the nocturnal decline in blood pressure after cardiac transplantation. Cyclosporine has been reported to induce fluid retention[9] and hence it may increase venous return during nighttime recumbency. An absent nocturnal decline in blood pressure has been reported

in patients with Cushing's syndrome and patients treated with high doses of glucocorticoids[10]. In addition, van de Borne and coworkérs have recently provided evidence for reappearance of the normal circadian blood pressure variation in cardiac transplant recipients after reduction of the dose of glucocorticoids.
The aim of the present study was to define the hemodynamic abnormalities underlying the absent or attenuated nocturnal fall in blood pressure in cardiac transplant recipients. Therefore the diurnal changes of ambulatory intra-arterial pressure and heart rate were measured in 23 cardiac transplant recipients and in 23 control subjects, matched for age and 24-hour mean arterial pressure. Relative diurnal changes in stroke volume, cardiac output and systemic vascular resistance were estimated using a pulse contour method.

Table 1. Diurnal Changes in Hemodynamic Variables in Cardiac Transplant Recipients With and Without Antihypertensive Medication

	With Antihypertensive Medication (n=6)	Without Antihypertensive Medication (n=17)
Mean Arterial Pressure (mmHg)		
24-Hour Average	101±7	107±14
Nocturnal Change	-3±12	-5±9*
Heart Rate (bpm)		
24-Hour Average	87±14	93±12
Nocturnal Change	-14±6†	-16±7‡
Nocturnal Change (%)		
Mean Arterial Pressure	-4±12	-5±8*
Heart Rate	-16±7†	-18±8‡
Stroke Volume	7±9	3±10
Cardiac Output	-8±9	-15±13‡
Vascular Resistance	3±6	11+10‡

Values are means±SD. Differences between the various hemodynamic parameters of the two subgroups were not significant. bpm, beats per minute; *, $p<0.05$, †, $p<0.01$, ‡, $p<0.001$ for changes within groups.

Subjects and Methods

Subjects
Twenty-three cardiac transplant recipients (18 males, five females), average age 42 years (range 18 to 56 years), who were all hemodynamically stable (cardiac index 3.7±0.8 L/min/m^2 (mean±SD), left ventricular ejection fraction 65±8%) gave their consent to participate in the study, which was approved by the Ethical Committee of the University

Hospital "Dijkzigt". The time interval between transplantation and intra-arterial blood pressure recording was 16±8 months (range 11 to 36 months). All subjects used cyclosporine (6.6±2.4 mg/kg per day) and prednisone (0.14±0.03 mg/kg per day) as maintainance immunosuppression. Six patients were receiving antihypertensive medication, which included nifedipine in 3, captopril in 2 and nifedipine and furosemide in 1 patient.
Invasive ambulatory blood pressure recordings in 23 subjects, matched for age and 24-hour mean arterial pressure, served as controls. These blood pressure recordings were obtained from our database of subjects, who were referred to us for assessment of their hypertension. The mean age of these subjects (22 males and one female) was 40 years (range 18 to 60 years). They did not use any medication for at least 3 weeks prior to the blood pressure recording.

Invasive 24-Hour Ambulatory Blood Pressure Recordings
Cardiac transplant recipients and control subjects were in the hospital at the time the blood pressure recordings were performed. Although the subjects were free to move within the hospital they were restricted with respect to the timing of meals and bed-time. In this way a certain degree of standardization over the registration period was obtained.
Invasive 24-hour ambulatory blood pressure was monitored according to previously described methods[11]. By means of the Seldinger technique a 10 cm long, 1.0 mm diameter Teflon catheter was introduced in the brachial artery of the non-dominant arm after local anaesthesia with a 2% lidocaine solution. The catheter was connected to a miniature perfusion-transducer device, which was fitted in front of the sternum at heart level. The transducer signal was recorded on magnetic tape by means of a portable tape recorder (Medilog Recorder II, Oxford Medical Instruments, Oxford, UK).
Registrations were read into an XP/7 computer system (Olivetti, Ivrea, Italy) with a sampling frequency of 160 Hz and a quantitation level of 12 bits. A dedicated computer program calculated pulse interval, and integrated mean arterial pressure (MAP) of individual beats. In addition, stroke volume (SV) was assessed by a corrected pulse contour method developed by Wesseling et al.[12,13]. The accuracy of this method has been established by comparison of cardiac output (CO) estimated by the pulse contour method with CO simultaneously estimated by thermodilution in patients undergoing a coronary bypass graft operation[14]. The two methods were well correlated ($r=0.94$; $n=64$), and the standard deviation of the differences between the methods against the mean of the methods was 10.6%. CO was calculated by the equation $CO=SV\times HR$. Total peripheral vascular resistance was calculated as $TPR=MAP/CO$, assuming a right atrial pressure of 0 mmHg throughout the 24 hours. Since stroke volume was not calibrated against a true value, stroke volume, cardiac output and total peripheral vascular resistance were only expressed as percentages of their 24-averages, which were set at 100%. For graphical presentation of the various hemodynamic parameters, 20-minute averages were calculated. As a measure of "short-term" as opposed to long-term or day-night variability of blood pressure and heart rate the standard deviation of these two parameters for each subsequent 20 minute period was computed. The diurnal variation of the hemodynamic variables was quantified as the difference between the average day and average nighttime values. Transience periods between day and night and night and day were removed by defining the day from 8:00 hours to 20:00 hours, and the night from 0:00 hours to 6:00 hours.

Statistics

Normal distribution of the parameters was verified as described by Shapiro and Wilk[15]. Since all parameters appeared to be normally distributed, values are presented as means±SD. The relationship between the various hemodynamic parameters is expressed by Pearson's correlation coefficient. To establish a statistical difference from zero and between groups, Student's unpaired and paired t-tests were used respectively. A p-value <0.05 (two-tailed) was regarded as significant.

Results

The 24-hour ambulatory blood pressure recordings appeared to be of good quality. In cardiac transplant recipients 96±4% and in control subjects 97±4% of all beats could be analyzed.

The 24-hour profiles of the absolute values of mean arterial pressure, heart rate and their short-term variabilities of cardiac transplant recipients and control subjects are shown in Figure 1. As compared to the controls, the nocturnal decline of blood pressure was considerably smaller in cardiac transplant recipients (respectively 5 versus 21 mmHg, Table 1). The 24-hour blood pressure profile of cardiac transplant recipients showed three prominent dips in blood pressure that coincided with the timing of meals. The 24-hour

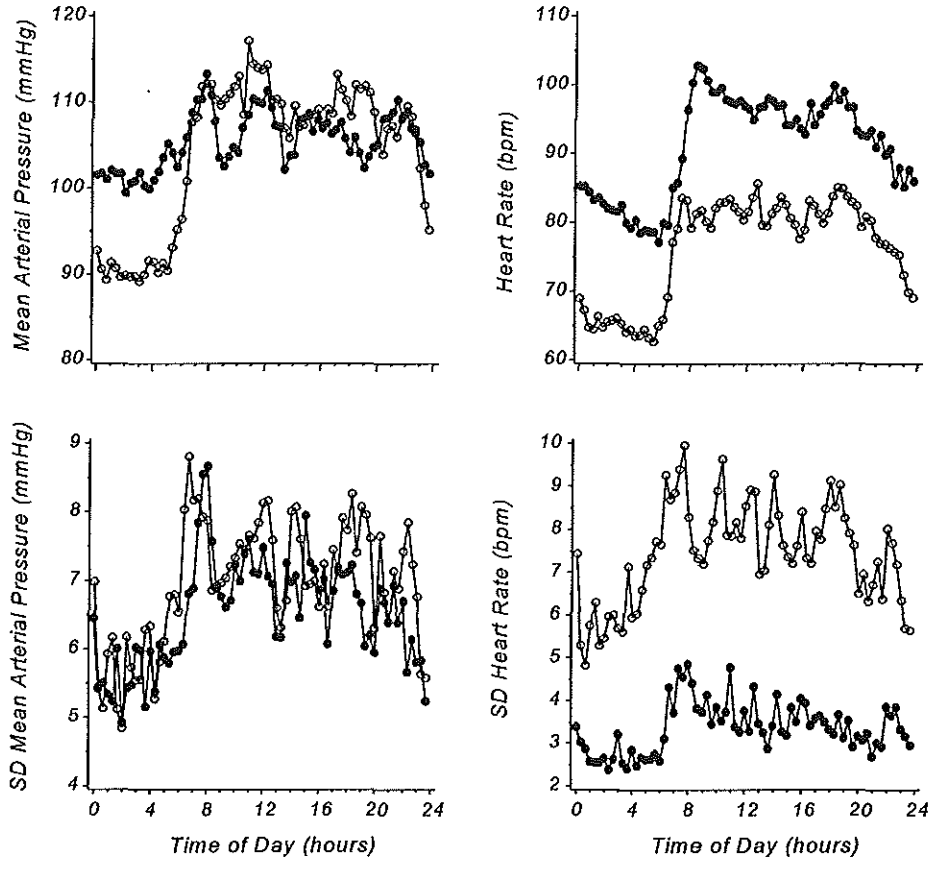

Figure 1. Twenty-four hour trend plots of mean arterial pressure, heart rate and their "short-term" variabilities in 23 cardiac transplant recipients and 23 control subjects. Twenty-minute averages of mean arterial pressure and heart rate are depicted, whereas the standard deviation of each 20 minute period of blood pressure and heart rate is presented as a measure of "short-term" variability of these two parameters. ●, Cardiac transplant recipients; ○, control subjects

blood pressure profile of control subjects showed a decrease in blood pressure in the early afternoon and a smaller decrease in blood pressure late in the evening. As expected, average 24-hour heart rate was considerably higher in cardiac transplant recipients than in control subjects, but the absolute difference in heart rate between day and night of the two groups was of the same magnitude (Table 1). As a consequence of the denervated state of the transplanted heart, the standard deviation of heart rate over each 20 minute period as a measure of short-term variability was considerably lower in cardiac transplant

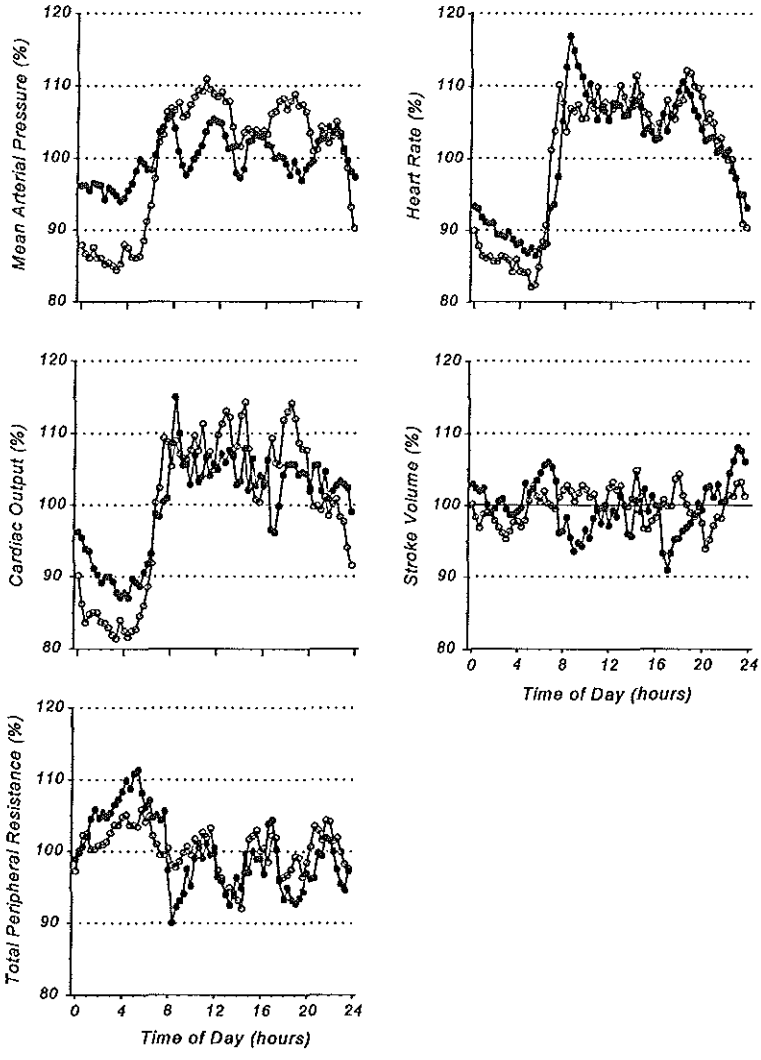

Figure 2. Twenty-four hour trend plots of the percentage changes of mean arterial pressure, heart rate, stroke volume, cardiac output and total peripheral vascular resistance in 23 cardiac transplant recipients and 23 control subjects. Twenty-minute averages of the various hemodynamic parameters are depicted. ●, *Cardiac transplant recipients;* ○, *control subjects.*

Table 2. Diurnal Changes of Hemodynamic Variables in Cardiac Transplant Recipients and Control Subjects

	Cardiac Transplant Recipients (n=23)	Control Subjects (n=23)	Statistical Significance of Difference Between Groups (p)
Mean Arterial Pressure (mmHg)			
24-Hour average	105±12	105±14	n.s.
Nocturnal change	-5±10*	-21±10‡	<0.001
Variability of Mean Arterial Pressure (mmHg)			
24-Hour average	6.5±0.9	6.9±0.9	n.s.
Nocturnal change	-1.3±1.0‡	-1.5±1.1‡	n.s.
Heart Rate (bpm)			
24-Hour average	92±13	75±9	<0.001
Nocturnal change	-16±7‡	-17±8‡	n.s.
Variability of Heart Rate (bpm)			
24-Hour average	3.4±1.2	7.4±1.6	<0.001
Nocturnal change	-0.8±0.8‡	-1.9±1.0‡	<0.001
Nocturnal Change (%)			
Mean arterial pressure	-5±9*	-20±8‡	<0.001
Heart rate	-18±8‡	-22±9‡	n.s.
Stroke volume	3±10	-3±11	n.s.
Cardiac output	-14±12‡	-24±13‡	<0.01
Vascular resistance	9±9†	4±13	n.s.

Values are means±SD. n.s. indicates not significant ($p \geq 0.05$); bpm, beats per minute; * $p<0.05$, † $p<0.01$, ‡ $p<0.001$ for changes within groups.

recipients than in control subjects both during the day and the night. The short-term variability of blood pressure in cardiac transplant recipients was of the same magnitude as in control subjects, and as in controls, it showed a diurnal variation with a lower value during the night than during the day (Figure 1).

Twenty-four hour profiles of the relative changes of mean arterial pressure, heart rate, stroke volume, cardiac output and total peripheral vascular resistance of cardiac transplant recipients and control subjects are shown in Figure 2. In control subjects the nocturnal fall in blood pressure was associated with a marked nocturnal fall in cardiac output. Nocturnal total peripheral vascular resistance as compared to the day-time value did not change (Table 1). The nocturnal fall in cardiac output was almost completely caused by a fall in heart rate and only a minimal, not significant, decrease in stroke volume. In cardiac transplant recipients the attenuated nocturnal fall in arterial pressure was associated with an attenuated nocturnal fall in cardiac output as compared to control subjects. In addition,

and in contrast to control subjects, nocturnal peripheral vascular resistance as compared to its daytime value was increased. The attenuated nocturnal fall in cardiac output in cardiac transplant recipients as compared to the control subjects was due to a slightly smaller nocturnal fall in heart rate and the tendency of a nocturnal increase in stroke volume (Table 1).

In order to assess possible effects of antihypertensive medication on the diurnal changes in the hemodynamic variables, nocturnal changes of mean arterial pressure, heart rate, stroke volume, cardiac output and systemic vascular resistance of the six cardiac transplant recipients who used antihypertensive medication at the time of the registration were compared with those changes of the 17 recipients without antihypertensive medication.

Although for none of the hemodynamic parameters differences were statistically significant, the nocturnal decrease in cardiac output tended to be even smaller in the patients using antihypertensive medication, predominantly because of a tendency for a nocturnal increase in stroke volume (Table 2).

Both in cardiac transplant recipients and in control subjects the nocturnal change in arterial pressure correlated with the nocturnal change in cardiac ouput ($r=0.72$; $p<0.001$ for cardiac transplant recipients and $r=0.45$; $p=0.03$ for control subjects), but not with the nocturnal change in total peripheral vascular resistance (Figure 3).

In cardiac transplant recipients the dips in blood pressure during the day were parallelled by decrements in peripheral vascular resistance. The dip in blood pressure in control subjects in the early afternoon was initially also associated with a decrease in vascular resistance and subsequently with a fall in stroke volume and cardiac output, whereas the smaller dip in blood pressure in the evening ran in parallel with a decrease in stroke volume.

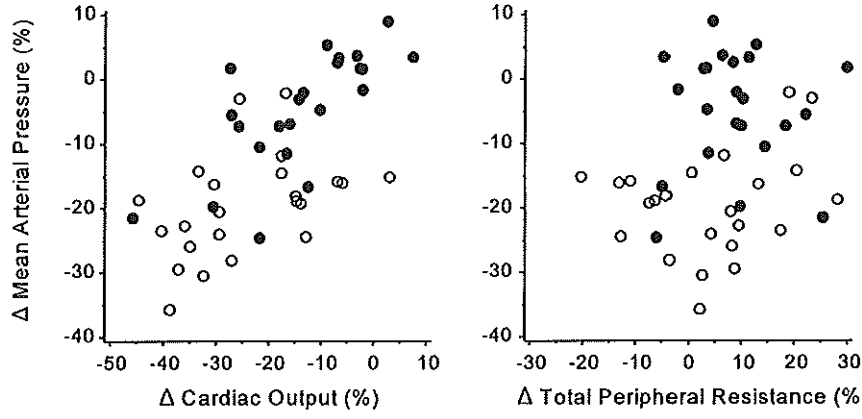

Figure 3. Scattergrams showing percentage nocturnal change in cardiac output or total peripheral vascular resistance versus percentage nocturnal change in mean arterial pressure in cardiac transplant recipients (●) and control subjects (○). Nocturnal changes in cardiac output and mean arterial pressure correlated: $r = 0.72$ ($p<0.001$) for cardiac tranplant recipients and $r = 0.45$; ($p=0.03$) for control subjects), whereas nocturnal changes in vascular resistance and mean arterial pressure did not correlate in either of the two groups.

Discussion

This study, using direct ambulatory blood pressure recordings, confirms the results of previous studies showing that the nocturnal fall of blood pressure is markedly attenuated in cardiac transplant recipients[1-6]. Hemodynamically this attenuation of the nocturnal fall in blood pressure is characterized by an attenuation of the nocturnal fall in cardiac output and a moderate increase in total peripheral vascular resistance.

The nocturnal changes in hemodynamics, i.e., a fall in cardiac output and an unchanged total peripheral vascular resistance, observed in our control subjects agree well with data reported in the literature in which cardiac output was measured either by indicator-dilution[16-18] or bioimpedance techniques[19]. This suggests that changes in stroke volume and hence cardiac output are estimated with reasonable accuracy with the pulse-contour method. Although a drawback of the pulse-contour method is that no absolute values, but only changes in stroke volume can be measured, this was not a handicap in the current study, since we were interested in within-subject changes in hemodynamics over the 24-hour period. Obviously, an important advantage of the pulse contour method, especially when applied to direct ambulatory blood pressure recordings, is that beat by beat information about systemic hemodynamic alterations can be obtained in unrestricted and undisturbed subjects outside the artificial environment of the cardiovascular laboratory.

The average nocturnal decrease in cardiac output was only 14% in cardiac transplant recipients as compared to 24% in control subjects. The tendency of a nocturnal increase in stroke volume as well as a somewhat smaller relative fall in heart rate accounted for this difference. The reason for this attenuated nocturnal fall in cardiac output remains uncertain, but probably the denervated state of the transplanted heart in this respect is most important. As a consequence of the denervated state the transplanted heart is not exposed to the normally occurring nocturnal increase in cardiac vagal and decrease in cardiac sympathetic tone. In addition, the effects of an increase in venous return on cardiac output during the night, is only indirectly and partially counteracted by cardio-pulmonary or baroreflex mediated changes in the activity of the autonomic nervous system, through changes in circulating catecholamines.

Besides the denervated state, the use of immunosuppressive therapy might also have contributed to the attenuated nocturnal fall in cardiac output. Cyclosporine is known to induce fluid retention[9] and hence it may increase venous return during nighttime recumbency, when extracellular fluid volume is shifted from peripheral to more central parts of the body. An attenuation of the circadian blood pressure variation in patients with Cushing's syndrome and in patients using glucocorticoids has been reported by Imai and coworkers[10]. However, in contrast to the present findings in cardiac transplant recipients, the attenuated circadian blood presure variation in patients treated with glucocorticoids is hemodynamically characterized by a marked nocturnal increase in total peripheral vascular resistance, whereas the normally occurring nocturnal decrease in cardiac output appears to be completely preserved[20]. It should further be remarked that the dose of glucocorticoids used by the patients studied by Imai and coworkers was several times higher than the dose of glucocorticoids used by the cardiac transplant recipients of this study.

Apart from the attenuated nocturnal decrease in cardiac output, another hemodynamic abnormality encountered in cardiac transplant recipients was a nocturnal increase in total peripheral vascular resistance. This nocturnal increase in vascular resistance is not easy to explain. To some extent it may be related to the fact that for the calculation of vascular resistance it was assumed that right atrial pressure remained stable at 0 mmHg throughout the 24-hour period. Since in normal subjects the diurnal variation of right atrial pressure as compared to the diurnal variation of mean arterial pressure is very low, the bias introduced by omitting diurnal changes in right atrial pressure is relatively small. However, for a subject with a relatively small nocturnal decrease of mean arterial pressure, that is typical for the cardiac transplant recipient, an increase of 1 mmHg of right atrial pressure during the night implies an overestimation of the nocturnal increase in vascular resistance by approximately 1%. Accordingly the nocturnal increase in vascular resistance is probably overestimated in our patients.

Besides the attenuated nocturnal fall in blood pressure the recordings of cardiac transplant recipients showed 3 prominent dips in blood pressure during the day. These dips in blood pressure coincided with the timing of meals and most likely are a direct consequence of it. Since our subjects were in the hospital at the time of the blood pressure recording the timing of meals was similar for each subject, thus synchronizing these postprandial dips in blood pressure in the groups. This may explain why the postprandial dips in blood pressure were so clearly visible on the blood pressure traces. Hemodynamically, the dips in blood pressure were associated with parallel decrements in total peripheral vascular resistance, most likely caused by arteriolar vasodilatation in the splanchnic circulation. In the control group the most marked fall in blood pressure during daytime occurred in the early afternoon, whereas small dips in blood pressure were seen in the morning after breakfast and in the evening. The prolonged dip in blood pressure in the early afternoon was due both to the consumption of the principal meal and a postprandial nap, which is customary in our hospital.

Postprandial dips in blood pressure have been described in eldery subjects and in patients with autonomic insufficiency[21,22]. In these subjects the postprandial dips in blood pressure are sometimes symptomatic and even may cause syncope. As far as we know symptomatic postprandial dips in blood pressure have not been reported yet in cardiac transplant recipients and also our patients noticed no hypotensive symptoms in relation to ingestion of food.

Although average daytime blood pressure was lowered by the postprandial dips in blood pressure this effect was far too small to account for the attenuated nocturnal fall in blood pressure. Without the dips in blood pressure, the estimated day-night difference in blood pressure increased form 5 to 7 mmHg, which is still considerably smaller than the 20 mmHg day-night difference in blood pressure observed in the control subjects.

The continuous recording of the intra-arterial blood pressure signal provided an opportunity to determine the "short-term" variability of blood pressure and heart rate in our subjects. In agreement with previous reports[23] short-term blood pressure and heart rate variability in control subjects, like blood pressure and heart rate, showed a clear diurnal variation with lower values during the night than during the day. As expected and related to the denervated state of the transplanted heart, short-term heart rate variability was markedly lower in cardiac transplant recipients than in control subjects. However, as in control subjects heart rate variability in cardiac transplant recipients also showed a diurnal

rhythm with a significantly higher value during the day than during the night. Most likely, a greater fluctuation in circulating catecholamines during the day related to different levels of activity explains the observed higher level of daytime heart rate variability. Alternatively, and in accordance with studies showing evidence for regional structural sympathetic reinnervation late after orthotopic cardiac transplantation[24,25], it could be that some of our patients had partial reinnervation of their transplanted heart. The short-term variability of blood pressure in cardiac transplant recipients was of the same magnitude as in the control subjects and, despite the attenuation of diurnal rhythm of blood pressure, its diurnal rhythm was comparable to that of control subjects. This finding indicates that to a certain extent the tonic level of blood pressure and its variability are regulated independently, which is in line with the results of a previously reported study by our group[26].

In conclusion, the nocturnal fall in blood pressure in cardiac transplant recipients is markedly blunted because of an attenuation of the nocturnal fall in cardiac output as well as a moderate increase in total peripheral vascular resistance. Apart from the attenuated nocturnal fall in blood pressure another peculiar finding in cardiac transplant recipients were marked postprandial dips in blood pressure. Finally, as opposed to the loss of long-term (day-night) blood pressure variability, "short-term" blood pressure variability is fully maintained in cardiac transplant recipients. "Unopposed" nocturnal increase in venous return may be one of the pathogenetic factors involved in the attenuated nocturnal fall in cardiac output and hence the attenuated nocturnal fall in blood pressure observed in cardiac transplant recipients. It seems worthwhile therefore to explore whether venodilatory treatment before patients go to sleep restores the normal diurnal rhythm. This is not ony of interest from a theoretical but also from a clinical point of view. Cyclosporine-induced hypertension occurs in the vast majority of cardiac transplant recipients[27,28]; an absent or attenuated nocturnal fall in blood pressure implies an important increase of the blood pressure load. This may contribute to the vascular and renal complications in these patients and plays a crucial role in the development of left ventricular hypertrophy[29,30]. A better understanding of the hemodynamic abnormalities underlying the attenuated blood pressure profile in cardiac transplant recipients might prove to be of value in the development of pharmacotherapeutic strategies that favourably influence long-term prognosis in these patients.

References

1. Reeves RA, Shapiro AP, Thompson ME, Johnsen AM: Loss of nocturnal decline in blood pressure after cardiac transplantation. Circulation 1986;73:401-408
2. Wenting GJ, van den Meiracker AH, Simoons ML, Bos E, Ritsema van Eck HJ, Man in 't Veld AJ, Weimar W, Schalekamp MADH: Circadian variation of heart rate but not of blood pressure after heart transplantation. Transplantation Proc 1987;19:2554-2555
3. Seheshed J, Thomas F, Thom M, Schifter S, Regitz V, Sheikh S, Oelkers W, Palm U, Meyer-Sabellek W, Hetzer R: Level and diurnal variations of hormones of interest to the cardiovascular system in patients with heart transplants. Am J Cardiol 1992;69:397-402

4. Angermann CE, Spes CH, Willems S, Dominiak WP, Kemkes BM, Theisen K: Zirkadianes Blutdruck- und Herzfrequenzverhalten nach orthotoper herztransplantation. Untersuchungen vor und während antihypertensiver Therapie. Z Kardiol 1989;78:228-235
5. Von Pölnitz A, Bracht C, Kemkes B, Höfling B: Circadian pattern of blood pressure and heart rate in the longer term after heart transplantation. J Cardiovasc Pharmacol 1990;15:S86-S89
6. Van de Borne P, Leeman M, Primo G, Degaute JP: Reappearance of normal circadian rhythm of blood pressure after cardiac transplantation. Am J Cardiol 1992;69:794-801
7. Mohanty PK, Thames MD, Arrowood JA, Sowers JR, McNamara C, Szentpetery S: Impairment of cardiopulmonary baroreflex after cardiac transplantation in humans. Circulation 1987;75:914-921
8. Scherrer U, Vissing SF, Morgan BJ: Cyclosporine-induced sympathetic activation and hypertension after heart transplantation. New Engl J Med 1990;323:693-699
9. Bantle JP, Boudreau RJ, Ferris TF: Suppression of plasma renin activity by cyclosporine. Am J Med 1987;83:59-64
10. Imai Y, Abe K, Sasaki S, et al: Altered circadian blood pressure rhythm in patients with Cushing's syndrome. Hypertension 1988;12:11-19
11. Miller-Graig MW, Haws D, Whittington J: New system for recording ambulatory pressure in man. Med & Biol Eng & Comp 1978;16:727-731
12. Wesseling KH, de Wit B, Weber JAP, Smith NT: A simple device for the continuous measurement of cardiac output. Adv Cardiovasc Phys 1983;5II:16-52
13. Smith NT, Wesseling KH, Weber JAP de Wit B: Preliminary evaluation of a pulse contour cardiac output method in man. Feasibility of brachial or radial arterial pressures. Proc San Diego Biomed Symp 1974;13:107-113
14. Jansen JCR, Wesseling KH, Settels JJ, Schreuder JJ: Continuous cardiac output monitoring by pulse contour during cardiac surgery. Eur Heart J 1990;11(Suppl 1):26-32
15. Shapiro SS, Wilk MB: An analysis of variance test for normality (complete samples). Biometrika 1965;52:591-611
16. Khatri IM, Freis ED: Hemodynamic changes during sleep. J Appl Physiol 1967; 22:867-873
17. Khatri IM, Freis ED: Hemodynamic changes during sleep in hypertensive patients. Circulation 1969;39:785-790
18. Lund-Johansen P: Hemodynamics in early essential hypertension. Acta Med Scand 1967; Suppl 482:1-101
19. Miller JC, Horvath SM: Cardiac output during human sleep. Aviat Space Environ Med. 1976;47:1046-1051
20. Imai Y, Abe K, Munakata M: Circadian blood pressure variations under different pathophysiological conditions. J Hypertension 1990;8(Suppl 7):S125-S132
21. Lipsitz LA, Nyquist RP, Wei JY, Rowe JW: Postprandial reduction in blood pressure in the eldery. N Engl J Med 1983;309:81-83

22. Lipsitz LA, Ryan SM, Parker A, Freeman R, Wei JY, Goldberger AL: Hemodynamic and autonomic nervous system responses to mixed meal ingestion in healthy young and old subjects and dysautonomic patients with postprandial hypotension. Circulation 1993;87:391-400
23. Mancia G, Ferrari A, Gregorini L, et al: Blood pressure and heart rate variabilities in normotensive and hypertensive human beings. Circ Res 1983;53:96-104
24. Wilson RF, Christensen BV, Simon A, Olivari MT, White CW, Laxson DD: Evidence for structural sympathetic reinnervation after human orthotopic transplantation. Circulation 1991;83:1210-1220
25. Wilson RF, Laxson DD, Christensen BV, McGinn AI, Kubo SH: Regional differences in sympathetic reinnervation after human orthotopic cardiac transplantation. Circulation 1993;88:165-171
26. Van den Meiracker AH, Man in 't Veld AJ, Ritsema van Eck HJ, Wenting GH, Schalekamp MADH: Determinants of short-term blood pressure variability. Effects of bedrest and sensory deprivation in essential hypertension. Am J Hypertens 1988;1:22-26
27. Thompson ME, Shapiro AP, Johnson AM, Itzkoff JM, Hardesty RL, Griffith BP, Balmson JM, McDonald RH, Hastillo A, Hess M: The contrasting effects of cyclosporine-A and azathioprine on arterial blood pressure and renal function following cardiac transplantation. Int J Cardiol 1986;11:219-229
28. Starling RC, Cody RJ: Cardiac transplant hypertension. Am J Cardiol 1990;65: 106-111
29. Verdecchia P, Schillaci G, Guerrieri M, Gatteschi C, Benemio G, Boldrini F, Porcellatti C: Circadian blood pressure changes and left ventricular hypertrophy in essential hypertension. Circulation 1990;81:528-536
30. Angermann CE, Spes CH, Willems S, Dominiak P, Kemkes BM, Theisen K: Regression of left ventricular hypertrophy in hypertensive heart transplant recipients treated with enalapril, furosemide, and verapamil. Circulation 1991;84:583-593

Chapter 11

Assessment of Diurnal Variation of Heart Rate and Blood Pressure in Cardiac Transplant Recipients by Square Wave Fitting

Introduction

There is some controversy about the presence of a normal circadian variation of blood pressure after cardiac transplantation. Although the first studies on 24-hour ambulatory blood pressure monitoring in cardiac transplant recipients reported an attenuation of the nocturnal decrease in blood pressure (see the references in Chapter 10), other investigators[1,2] reported a return to a normal circadian variation in blood pressure in months after transplantation. Our own study[3], using continuous intra-arterial blood pressure measurement, demonstrated an attenuated circadian variation of blood pressure up to three years after cardiac transplantation, using fixed time periods to calculate the night-day difference of blood pressure. In the present study, we use this material to investigate the applicability of the square wave fit for the detection of abnormalities in circadian variation of heart rate and blood pressure.

Subjects and Methods

For the present study, the blood pressure registrations recorded for the study in Chapter 10 were used[3]. These intra-arterial ambulatory registrations were obtained in 23 cardiac transplant recipients, 11 to 36 months after transplantation, and in 23 control subjects matched for age and 24-hour mean arterial pressure. Six of the cardiac transplant recipients received antihypertensive medication at the time of the registration. During the blood pressure recordings, the subjects were hospitalized to standardize environmental conditions. From these recordings, 24-hour profiles consisting of 20-minute averages of heart rate and mean arterial pressure were constructed. The diurnal variation in the individual profiles was modeled by restricted square wave fitting as described in Chapter 7.

To detect abnormalities in the diurnal variation of mean arterial pressure in individual cardiac transplant recipients, a reference area for normal phase and magnitude of the circadian variation of blood pressure was constructed from the square wave parameters of the control subjects. Since t_{up} describes the phase of circadian variation more precisely than t_{down}, as was demonstrated in Chapter 7, t_{up} was chosen as the parameter to represent phase, whereas cc_{max} and the amplitude were chosen to represent the magnitude of the diurnal variation.

Statistics

Statistical calculatations on clock time values were performed by means of the techniques for directional data as described in Chapter 6. For the other values, the statistical significance of differences between groups was assessed by unpaired t-tests. The number of significant fits were compared by a Fisher exact test[4]. Correlation between the square wave amplitudes and the 24-hour average levels of heart rate and mean arterial pressure was calculated as the Spearman rank correlation coefficient. For the assessment of statistical significance, a 0.05 significance level was chosen. All statistical computations

were perfomed with SAS statistical software (SAS V6.04 for Personal Computers, SAS Institute Inc., Cary, North Carolina, USA).

Results

Modeling of Profiles

The results of restricted square wave fitting in the profiles of heart rate and mean arterial pressure are displayed in Table 1. In the control subjects, there was a positive correlation between the 24-hour average levels of heart rate and blood pressure and the square wave amplitudes of these parameters. In the cardiac transplant recipients, such a relation could

Table 1. Results of Restricted Square Wave Fitting in 24-Hour Profiles of Heart Rate and Mean Arterial Pressure in Cardiac Transplant Recipients and Control Subjects

	Cardiac Transplant Recipients (n=23)	Control Subjects (n=23)	Significance of Difference between Groups (p)
Heart Rate			
24-Hour average (bpm)	92±12.9	75±9.3	<0.0001
Amplitude of diurnal variation (bpm)	15.3±5.5	17.0±6.6	n.s.
t_{up} (hours)	7:34 [0.92] *	7:22 [0.92] *	n.s.
cc_{max}	0.77±0.10	0.75±0.11	n.s.
No. of significant fits	22	21	n.s.
Correlation coefficient of amplitude with 24-hour average	0.02	0.64 †	
Mean arterial pressure			
24-Hour average (mmHg)	105±12.3	105±13.6	n.s.
Amplitude of diurnal variation (mmHg)	12.3±6.0	20.4±8.3	<0.001
t_{up} (hours)	5:51 [0.20]	6:45 [0.89] *	n.c.
cc_{max}	0.62±0.13	0.78±0.14	<0.0001
No. of significant fits	16	21	n.s.
Correlation coefficient of amplitude with 24-hour average	0.11	0.51 ‡	

Values are mean±SD or mean directional vector time [mean directional vector length].
*, $p<0.05$ for mean directional vector time; †, $p<0.001$; ‡, $p< 0.05$ for Pearson correlation coefficient; n.s., non-significant for difference between groups, n.c. not calculated because of non-significant value in one group.

not be demonstrated. Therefore, the comparison of the amplitudes between the two groups is based on the absolute values of the amplitude rather than the amplitudes expressed as a percentage of the 24-hour average.

In most profiles of heart rate of both control subjects and cardiac transplant recipients, the diurnal varation was adequately modeled, as was judged by visual inspection (data not shown). This explains the high values of cc_{max}, the parameter that represents the fraction of the variation in the 24-hour profile that is contained in the square wave model, and the high proportion of significant fits (22 and 21 out of 23 profiles for cardiac transplant

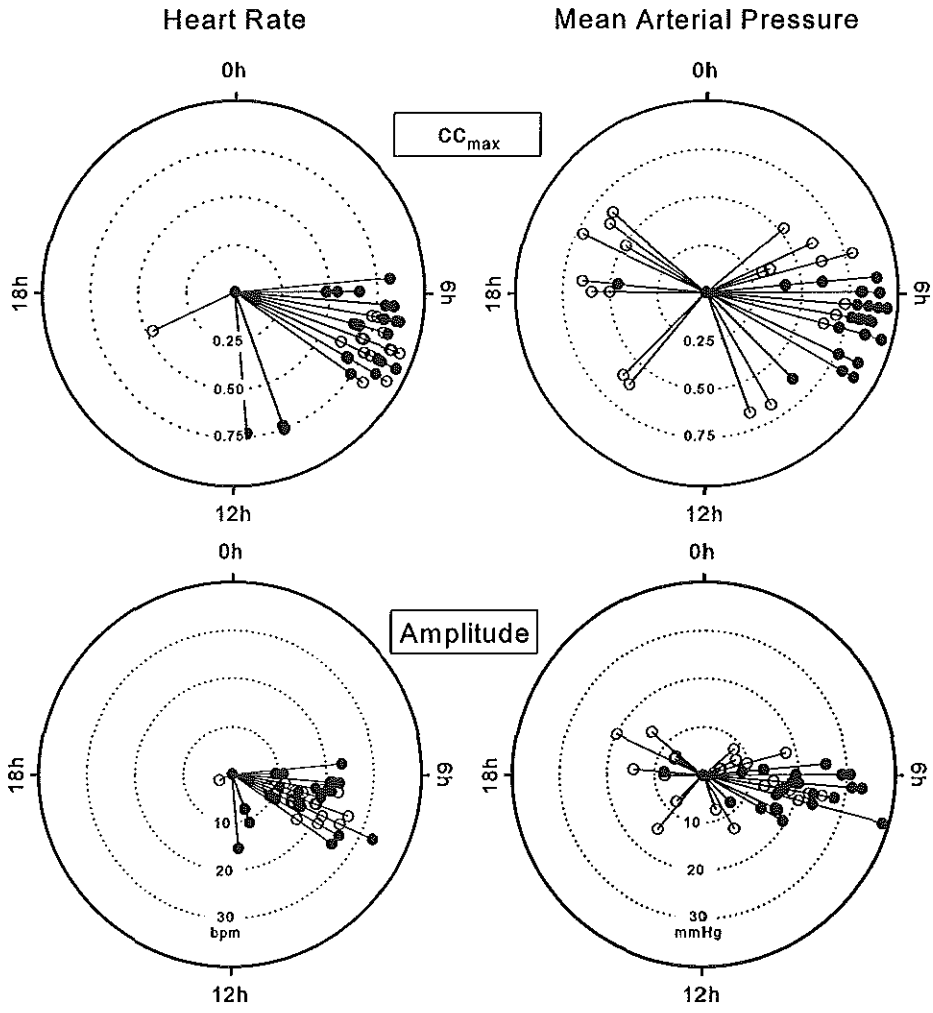

Figure 1. Circular plots represent the diurnal variation of heart rate (left panels) and mean arterial pressure (right panels) as assessed by restricted square wave fitting in 23 control subjects (●) and 23 cardiac transplant recipients (○). The angle with the vertical axis represents t_{up}, and the distance to the centre of the circle represents cc_{max} (upper panels) or amplitude (lower panels).

recipients and control subjects, respectively). As expected, the average 24-hour heart rate is markedly higher in the cardiac transplant recipients than in the control subjects. The nocturnal change in heart rate as calculated by the square wave amplitude, however, is approximately identical in the two groups. The values for t_{up} of the heart rate profiles are almost identical for cardiac transplant recipients and control subjects, as is expected since the environmental condidtions were standardized in the subjects. Between the individuals of both groups, however, there is a considerable scatter.

For the profiles of mean arterial pressure, there were marked differences between the cardiac transplant recipients and the control subjects. Since the subjects were matched for blood pressure, the values of 24-hour mean arterial pressure are almost identical in both groups. In the control subjects, the average value of t_{up} in the profiles of mean arterial pressure is about 30 minutes earlier than in the heart rate profiles. This difference, however, does not reach statistical significance. The average square wave amplitude, the average value of cc_{max} as well as the number of significant fits, are all considerably smaller in the cardiac transplant recipients than in the control subjects. In contrast to the control subjects, the average value of t_{up} in the cardiac transplant recipients is non-significant, i.e., the distribution of t_{up} cannot be discerned from a random distribution. This implies that in this group, a preferential time for t_{up}, and thereby a synchronized diurnal blood pressure rhythm, cannot be demonstated.

Figure 1 displays the relation between the phase and the magnitude of the diurnal variation in profiles of heart rate and mean arterial pressure of both groups. The individual observations are somewhat better visible in the plots that use cc_{max} to represent the magnitude of the diurnal variation, since most of these observations are further from the centre of the circle and therefore scattered over a wider area that the observations that use the amplitude. It appears that the amplitude and cc_{max} convey almost identical information, and

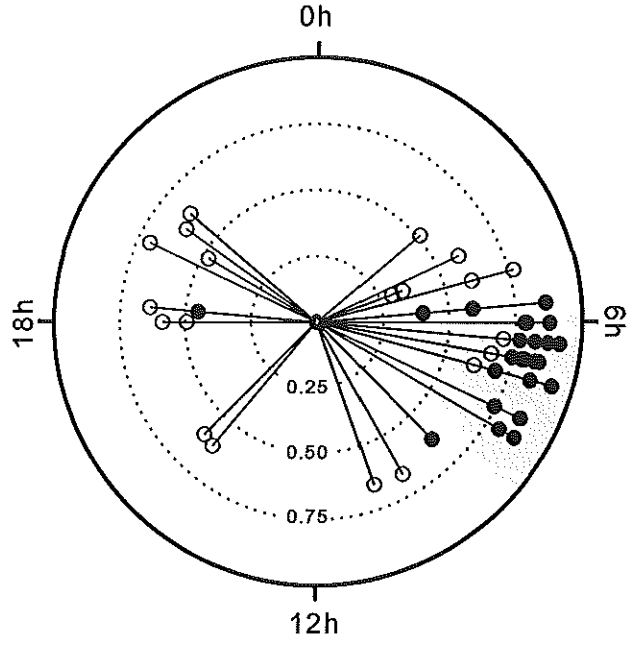

Figure 2. (Same data as in upper right panel of Figure 1.) Circular plot shows t_{up} and cc_{max} obtained by restricted square wave fitting in profiles of mean arterial pressure of 23 control subjects (●) and 23 cardiac transplant recipients (○). The value of t_{up} is represented by the angle with the vertical axis, and the value of cc_{max} by the distance to the centre of the unit circle. One of the control subjects has a clearly abnormal pattern of circadian variation. The distribution of the other 22 control subjects is used to define the area of normal circadian variation.

therefore only the plots that use cc_{max} are discussed. In the profiles of heart rate, most observations have values of t_{up} in the range from 5:40 to 8:20 hour, with exception of three control subjects (values for t_{up} are 10:40, 10:40 and 11:40 hour, one observation is not visible because of overlapping) and one cardiac transplant recipient (t_{up} at 16:40 hour).

Abnormal Circadian Variation of Mean Arterial Pressure
In Figure 2, the individual values of t_{up} and of cc_{max} calculated from the profiles of mean arterial pressure are displayed in detail. Of the 23 control subjects, 22 have values of t_{up} in the range of 5:40 to 9:00 hour. One control subject, however, has a clearly distinct value for t_{up} (18:20 hour) which is approximately 12 hours later than the average in the control group, i.e., the subject is in anti-phase with the other control subjects. The subject appears to have a relatively small value of cc_{max}, and thus the square wave fit in this subject is non-significant. If this clearly outlying subject is omitted from the control group, the area in which the distribution of the remaining control subjects is contained may be considered as a reference area for a normal circadian variation. Of the cardiac transplant recipients, six subjects are within this area (three are not visible because of overlap) and are thus classified as having a normal phase and amplitude of the diurnal variation of blood pressure, whereas the other 17 are classified as abnormal.

Discussion

Modeling of Profiles
In the present study, diurnal variation of heart rate and mean arterial blood pressure in cardiac transplant recipients and hypertensive subjects was assessed by means of the square wave fit. In both groups, the circadian variation of heart rate was modeled satisfactorily by a square wave according to visual inspection and fraction of significant fits. A diurnal rhythm of heart rate was demonstrated in both groups by the presence of a preferential time for t_{up}. Despite a higher baseline value of heart rate in cardiac transplant recipients, the diurnal rhythm as modeled by the square wave fit in this group was almost identical to the rhythm in the control subjects.
According to visual inspection and the fraction of significant fits, the diurnal variation in the profiles of mean arterial pressure recorded in control subjects was adequately modeled by the square wave fit, and a diurnal blood pressure rhythm could be demonstrated, with t_{up} not significantly different from the value measured in the heart rate profiles. In the group of cardiac transplant recipients, the rhythm was not statistically significant.

Correspondence of Square Wave Amplitude to Nocturnal Change in Blood Pressure
In Chapter 9, we have calculated the nocturnal change of blood pressure and heart rate in these groups over fixed time periods. For this purpose, the night was defined as the period from 0:00 to 6:00 hour and the day as the period from 8:00 to 20:00 hour. The nocturnal changes thus calculated for heart rate (-16±7 bpm in cardiac transplant recipients and -17±8 bpm in control subjects) and for mean arterial pressure in the control subjects (-21±10 mmHg) are in accordance with the square wave amplitudes in the present study (15.3±5.5 bpm, 17.0±6.6 bpm, and 20.4±8.3 mmHg, respectively). The nocturnal change of mean arterial pressure according to fixed clock times was -5±10 mmHg in the cardiac

transplant recipients, whereas the amplitude in these profiles as assessed by square wave fitting was 12.3±6.0 mmHg. In agreement with our Monte Carlo experiments in Chapter 6, a possible explanation for this overestimation is that the amplitude of the diurnal blood pressure variation is small in comparison with the 20-minute to 20-minute blood pressure variation. If it is assumed that the present group of cardiac transplant recipients has a normal phase of the circadian blood pressure variation and that only the amplitude is attenuated, the square wave amplitude may be estimated from the fixed periods that were used to define the day and night. Under this assumption, the square wave amplitude is 5 mmHg, whereas the standard deviation over the day and night periods are 8.9±3.7 mmHg and 5.3±1.8 mmHg, respectively, resulting in an amplitude to noise ratio (AN-ratio) of about 0.7. From Figure 2 in Chapter 6, it appears that for an AN-ratio of 0.7, an overestimation of the amplitude of 5 mmHg can be expected, which would approximately explain the overestimation of the nocturnal change of blood pressure by the square wave fit found in our cardiac transplant recipients (12.3 vs 5 mmHg). For an AN-ratio of 0.7, however, the percentage of significant fits is approximately zero, as can be read from Figure 5 in Chapter 6. Since we found 16 out of the 23 fits significant, the assumption that only an attenuated night-day difference underlies the abnormal nocturnal variation must be wrong. A different explanation may be that the phase of the diurnal variation is disturbed. Indeed, seven cardiac transplant recipients had a blood pressure profile that was in antiphase with the control subjects, as is visualized in Figure 2 (subjects with t_{up} in the range from 18:00 to 21:00 hours). Clearly, in these subjects the nocturnal blood pressure change calculated over the fixed periods as defined above is reversed, which attenuates the average nocturnal decrease in blood pressure. In the square wave model, however, the phase of the fit in these seven subjects is reversed, whereas the amplitude is approximately identical to the other cardiac transplant recipients. Inversion of the phase was also demonstrated by Van de Borne and co-workers[2] using periodogram modeling in cardiac transplant recipients. Such abnormalities of 24-hour blood pressure variation may remain undetected if the assessment of the diurnal blood pressure variation is based on the calculation of the nocturnal change of blood pressure, whether the calculations are based on diaries or fixed time periods.

Cyclosporine

In our previous studies[3,5] we hypothesized that the attenuation of the nocturnal decrease in blood pressure in our cardiac transplant recipients may have been caused by the use of cyclosporine. This medication induces fluid retention, and may therefore increase venous return during nighttime recumbency. In cardiac transplant recipients that received lower dosages of this immunosuppressivum, a normal diurnal variation of blood pressure has been reported[1,2]. Further support for this hypothesis was recently provided by van den Dorpel and co-workers[6]. These investigators reported an attenuated circadian variation of blood pressure in renal transplant recpients receiving cyclosporine and prednisone as immunosuppressive medication. The circadian variation of blood pressure was partially restored when the cyclosporine was replaced by azathioprine. It is therefore likely that cyclosporine alone or in combination with prednisone may attenuate the diurnal blood pressure variation.

Conclusion

We have modeled the diurnal variation of heart rate and blood pressure by means of restricted square wave fitting. In accordance with our previous studies[3,5] we have demonstrated an intact diurnal rhythm of heart rate and an attenuation of the diurnal variation of blood pressure in cardiac transplant recipients in comparison to control subjects by this modeling technique. In addition we demonstrated that seven of the 23 cardiac transplant recipients were in antiphase with the control subjects. We conclude that the square wave fit may be used to detect an abnormal circadian variation of blood pressure. The clinical relevance of the inversion of the phase of the circadian blood pressure variation needs further investigation.

References

1. Von Pölnitz A, Bracht C, Kemkes B, Höfling B: Circadian pattern of blood pressure and heart rate in the longer term after heart transplantation. J Cardiovasc Pharmacol 1990;15:S86-S89
2. Van de Borne P, Leeman M, Primo G, Degaute JP: Reappearance of a normal circadian rhythm of blood pressure after cardiac transplantation. Am J Cardiol 1992;69:794-801
3. Idema RN, van den Meiracker AH, Balk AHMM, Bos E, Schalekamp MADH, Man in 't Veld AJ: Abnormal diurnal variation of blood pressure, cardiac output, and vascular resistance in cardiac transplant recipients. Circulation 1994;90:2797-2803
4. Diem K, Lentner C, eds: Wissenschaftliche Tabellen, 7th edition. J.R. Geigy A.G., Pharma, Basle, Switzerland, 1986, p. 117, p. 123
5. Idema RN, van den Meiracker AH, Balk AHMM, Bos E, Schalekamp MADH, Man in 't Veld AJ: Decreased circadian blood pressure variation up to three years after heart transplantation. Am J Cardiol 1994;73:1006-1009
6. Van den Dorpel MA, van den Meiracker AH, Lameris ThW, Boomsma F, Levi M, Man in 't Veld AJ, Weimar W, Schalekamp MADH: Cyclosporine A impairs the nocturnal blood pressure fall in renal transplant recipients. Hypertension 1996 (in press)

Chapter 12

Summary

Summary

Chapter 1 describes the rationale of ambulatory blood pressure monitoring and the increasing insight in the relevance of the attenuation of the normal nocturnal decrease in blood pressure as a marker of cardiovascular disease. Despite this well-recognized relevance, there is still no consensus regarding how to define and calculate the nocturnal blood pressure fall, and many different methods have been used in various studies.
Since the nocturnal decrease in blood pressure is the most prominent and constant feature of the 24-hour blood pressure profile, the present thesis investigates whether this feature can be calculated directly from the blood pressure profile without using additional information. For this purpose, a square wave model of diurnal blood pressure variation is developed and evaluated. The following questions are investigated:

- What is the performance of the blood pressure recording system and analysis software?
- How does the square wave model compare to other models of circadian blood pressure variation, and which model best describes the circadian rhythm?
- What is the dependency of the square wave parameter estimation on the measurement frequency, the between-measurement variation and the magnitude of the diurnal blood pressure variation?
- Can the square wave fit be applied to 24-hour blood pressure registrations obtained by means of non-invasive measurement, and can it be used in outpatients in whom daily activities are less restricted than in hospitalized subjects?
- Does the square wave fit estimation of the period of low pressure correspond with the period of bed rest?
- What is the hemodynamic basis of the attenuated circadian variation of blood pressure in cardiac transplant recipients?

Chapters 2 describes the blood pressure recording system used in most of the present studies. The Oxford Medilog blood pressure recording system allows continuous intra-arterial recording of the blood pressure signal. The main sources of measuring error are drift and signal damping. Because of the design of our studies, the quantitative effect of drift on the study results is very small. The quantitative effect of signal damping is limited by the fact that only within-recording changes were measured.

Chapter 3 describes and evaluates the software used for the beat-to-beat analysis of the blood pressure signal. The sensitivity and specificity of the beat detection are evaluated on the basis of time ratios. The sensitivity was satisfactory with a median value of 99.3%. The specificity is smaller (median value 93.5%; range 51.2% to 100%), but since the largest part of the signal is free of artifacts, the number of erroneously detected beats is always small in comparison with the the total number of beats detected (<5%). The errors of the analysis algorithm are negligible for the blood pressure and heart rate statistics

calculated in the present studies. The average error of the the nocturnal change of stroke volume, cardiac output, and total peripheral resistance are larger but small in comparison with the nocturnal change in these parameters.

In **Chapter 4**, we develop a simple mathematical model of diurnal blood pressure variation which nevertheless does justice to the specific form characteristics of individual blood pressure registrations. The model is evaluated for intra-arterial measurements of blood pressure obtained in hospitalized patients with mild to moderate untreated essential hypertension. Inspection of the profiles suggests a model of blood pressure consisting of two contiguous, complementary periods of constant pressure, a so-called square wave. Determination of the times of transience between both periods (segmentation) is performed individually, using a least square error criterion. Results are compared to those obtained by conventional methods including a segmentation method based on Fourier modeling. The square wave fit accounts for a larger fraction (66%) of circadian variance of mean arterial pressure than modeling based on segmentation by visual inspection (59%, considerable observer bias) or by clock time (50%). Application of the Minnesota Cosinor method results in the poorest description (47%). Segmentation based on harmonic modeling (61%) appears to be cumbersome because of the large number of harmonics needed, and the significance of additional information offered over the square wave fit is dubious. Observer bias makes segmentation by visual inspection unsuitable for assessment of the circadian variance of blood pressure. Even when daily activities are strictly regulated such as in a hospital environment, circadian variance appears to be modeled considerably better by the clock time independent segmentation methods than by clock time. As compared to harmonic analysis, square wave fitting is simple, and it appears to best model the circadian variance.

In **Chapter 5**, we comment on the periodogram method as it was introduced for the modeling of diurnal blood pressure variation. We state that the bimodal distribution of blood pressure during the day-time that is postulated by its authors cannot be concluded from application of the method presented. Instead, the nocturnal decrease in blood pressure is likely to induce a bimodal distribution of blood pressure during the day-time as an artifact in their model.

The precision and accuracy of the estimation of the square wave parameters in a 24-hour profile depend on the number of samples in the profile and therefore on the sampling frequency. The quality of the fit is also dependent on the variation that is contained in the diurnal variation in comparison with the variation from other sources, such as the measuring error and the short-time blood pressure variation, i.e., the signal-to-noise ratio. This is of particular importance in non-invasive measurement, which combines a limited measuring frequency with a larger imprecision when compared to intra-arterial measurement. These dependencies are investigated in **Chapter 6**. By means of Monte Carlo experiments in simulated blood pressure profiles, we investigate the dependency of the square wave amplitude and transience times on the sampling frequency, ratio between the amplitude of the diurnal variation and the noise, and the length of the high and low blood pressure period. If the ratio of the amplitude of the diurnal variation and the standard deviation of the noise is at least 1.5, i.e., in most profiles of healthy subjects, a

measuring frequency of 2.5/hour should suffice for the assessment of the square wave amplitude. If this ratio is 1.0, a measuring frequency of at least 6.0/hour should be used. Under these circumstances, the bias of the amplitude does not exceed 0.5 mmHg, whereas the additional imprecision caused by the square wave fit over the sample size related imprecision is smaller than 0.6 mmHg. If the ratio is smaller than 1.0, assessment of the amplitude is positively biased and the square wave fit cannot be used to assess the amplitude of the diurnal variation. Whether in a group of profiles the diurnal variation is sufficiently large for application of the square wave fit can be assessed by calculating the fraction of significant fits. The square wave transience times are less dependent on the magnitude of the diurnal blood pressure variation than the square wave amplitude. If the diurnal variation is not sufficient for amplitude assessment, information on the phase of the diurnal variation may therefore in some cases still be obtained by means of the calculation of the transience times.

In **Chapter 7** we investigate the applicability of the square wave fit in blood pressure profiles recorded by means of cuff measurement in healthy outpatients. In comparison with intra-arterial blood pressure measurement, these registrations are characterized by larger short-term blood pressure fluctuations and a smaller number of measurements. The diurnal variation is modeled well by square wave fitting, and there is a good correspondence between the modeled transience times and the period of bed rest. These results are obtained after a modification of the fitting technique, which restricted the length of the low period to the expected range, i.e., to a range of 5 to 13 hours. The subjects have a wide range of bed rest periods according to their diaries, and we postulate that the change of blood pressure during this period is therefore probably better assessed by square wave fitting than by clock time dependent methods, a hypothesis that has been confirmed by recent work of other investigators. In our material, a significant square wave fit approximately corresponds to a nocturnal decrease in blood pressure of 10 mmHg or 10%, which is by convention referred to as a "dipping" profile.

In **Chapter 8** we illustrate the limitations of the square wave model in renal transplant recipients before and after conversion from cyclosporine to azathioprine immunosuppression. In a previous study it was demonstrated that during cyclosporine medication, the diurnal variation of blood pressure was attenuated in comparison with azathioprine immunosuppression. During both immunosuppressive regimens, the diurnal variation of blood pressure is insufficient for a quantitative assessment of diurnal variation by means of the square wave fit. As a consequence, the square wave fit cannot demonstrate a difference in the magnitude of the diurnal variation between the regimens. Differences between the immunosuppressive regimens are found, however, by means of the distribution of the transience times.

In **Chapters 9, 10** and **11**, the hemodynamic characteristics of cardiac transplant recipients are investigated by means of direct ambulatory blood pressure measurement. Cardiac output, stroke volume, and total peripheral resistance are assessed by means of a corrected pulse contour method. In accordance with other investigators, we find an increase in 24-hour blood pressure and heart rate up to three years after transplantation. The elevation of blood pressure is probably caused by the cyclosporine used by these

patients. This immunosuppressant is known to cause fluid retention and an increase in sympathetic nerve activity, and hypertension is a well-known side effect of this drug. The increase in heart rate can probably be attributed to the denervated state of the transplanted heart, in particular absence of vagal tone.

Modeling of circadian variation based on fixed time periods demonstrates a normal diurnal variation of heart rate, whereas the diurnal variation of blood pressure is attenuated. This attenuation is partially explained by a decrease in blood pressure during the day caused by three short-lasting dips in blood pressure, probably as a consequence of food ingestion. In some profiles of cardiac transplant recipients, the diurnal blood pressure rhythm appears to be reversed. Hemodynamically, the attenuated fall in nocturnal blood pressure is characterized by an attenuated decrease of cardiac output. This relative increase in nocturnal cardiac output might be explained by the increase in venous return during nighttime recumbency, which cannot be compensated for by means of a decrease in cardiac inotropy and chronotropy. A restraint of sympathetic outflow to the vascular tree due to an interruption of the ventricular-baroreceptor reflex after heart transplantation could be a contributory factor to the nocturnal mismatch between cardiac output and vascular resistance.

Immunosuppression most likely also plays a role in the attenuation of the diurnal blood pressure variation after cardiac transplantation. Since cyclosporine causes both fluid retention and an increase in sympathetic nerve activity, it may enhance both aforementioned mechanisms. The study in renal transplant recipients demonstrates that cyclosporine may also attenuate the nocturnal fall in blood pressure independently of cardiac denervation. The possible role of cyclosporine is corroborated by studies in which a restoration of the normal circadian blood pressure variation after cardiac transplantation is reported, since the subjects in these studies used a lower cyclosporine dosage than the subjects in our study. Since cardiac transplant recipients are at an increased risk of cardiovascular complications, which may be related to the 24-hour blood pressure load, a pharmacological strategy aimed at reduction of both the cyclosporine dosage and the venous return during the night may be of possible value in the improvement of long-term prognosis in these patients.

In conclusion, we have introduced a simple technique that models diurnal blood pressure variation by segmenting the 24-hour blood pressure profile in a period of high and a period of low pressure. The method can detect the presence or absence of a diurnal blood pressure rhythm, and provide quantitative information on the amplitude and the transience times. If the circadian variation is attenuated, the method does not estimate the amplitude reliably but it can in some cases still provide information on the phase of the rhythm.

Questions that need further investigation are:

- Can the nocturnal decrease in blood pressure be reliably calculated by segmenting a blood pressure profile in a day-time and a night-time period using the transience times calculated from the corresponding heart rate profile? This question is of particular importance if the circadian blood pressure variation is too small for direct calculation of the square wave amplitude from the 24-hour blood pressure profile.
- Can the robustness of the square wave fit be improved by optimizing criteria other than the cross correlation coefficient? Such a technique would decrease the necessity of an outlier rejection procedure.
- The most important question concerns determination of the clinical importance of the square wave fit amplitude in comparison with other measures of circadian blood pressure variation with relation to target organ damage, morbidity and mortality. These investigations may include re-analysis of data in published studies.

Chapter 13

Samenvatting

Samenvatting

Hoofdstuk 1 beschrijft de rationale van ambulante bloeddrukmeting, en het groeiende inzicht in de relevantie van een verminderde nachtelijke bloeddrukdaling als een indicator van cardiovasculaire ziekte. Hoewel deze relevantie algemeen onderkend wordt, bestaat er toch geen consensus over de wijze waarop de nachtelijke bloeddrukdaling gedefiniëerd en berekend moet worden. In de diverse studies over dit onderwerp worden hiervoor dan ook verschillende methoden gehanteerd.
De nachtelijke bloeddrukdaling is de meest in het oog springende karakteristiek van een 24-uurs bloeddrukprofiel. In dit proefschrift wordt onderzocht of hiervan gebruik kan worden gemaakt om de nachtelijke bloeddrukdaling rechtstreeks uit de vorm van het 24-uurs bloeddrukprofiel te berekenen.
Voor dit doel wordt een blokgolfmodel van de 24-uurs bloeddrukvariatie ontwikkeld en geëvalueerd. De volgende vragen werden hierbij onderzocht:

- Wat zijn de karakteristieken van het bij de studies gebruikte meetsysteem en van de programmatuur die is gebruikt voor de analyse van het geregistreerde signaal?
- Hoe verhoudt het blokgolfmodel zich tot bestaande modellen van 24-uurs bloeddrukvariatie, en welk van deze modellen beschrijft de 24-uurs bloeddrukvariatie het best?
- Wat is de invloed van de frequentie waarmee de bloeddruk wordt gemeten, de bloeddrukvariatie tussen deze metingen, en de grootte van de nachtelijke bloeddrukdaling op de betrouwbaarheid waarmee de blokgolfmethode de 24-uurs bloeddrukvariatie meet?
- Kan het blokgolfmodel worden toegepast op niet-invasieve 24-uurs bloeddrukregistraties, en op poliklinische metingen?
- Komt de door het blokgolfmodel aangegeven periode van lage bloeddruk overeen met de periode van nachtrust?
- Wat is de hemodynamische basis van de verminderde nachtelijke bloeddrukdaling die na harttransplantatie wordt gevonden?

In **hoofdstuk 2** wordt het bloeddrukmeetsysteem dat we in enkele studies gebruikt hebben beschreven. Met het Oxford Medilog systeem kan bij de ambulante patiënt de bloeddruk continu intra-arteriëel gemeten worden. De belangrijkste fouten in de meting worden veroorzaakt door drift en demping van het signaal. Door het ontwerp van de studies is het kwantitatieve effect van de drift zeer gering. Het kwantitatieve effect van signaaldemping wordt beperkt door het feit dat alleen verschillen binnen dezelfde bloeddrukregistratie worden gemeten.

Hoofdstuk 3 beschrijft en evalueert de software die is gebruikt om het bloeddruksignaal van hartslag tot hartslag te analyseren. De sensitiviteit en specificiteit van het detectie-algoritme worden geëvalueerd aan de hand van de lengtes van het geclassificeerde signaal.

De sensitiviteit was bevredigend; de mediane waarde was 99.3%. De specificiteit was kleiner, met een mediane waarde van 93.5% en een bereik van 51.2% tot 100%. Doordat weinig artefacten in het signaal voorkomen, is het signaal dat ten onrechte gebruikt wordt in de analyse desondanks gering (<5% van het totaal). De fouten in de studie-uitkomsten van bloeddruk en hartfrequentie blijken verwaarloosbaar klein te zijn. De gemiddelde fout in de berekening van het slagvolume, de cardiac output, en de perifere vaatweerstand zijn groter, maar blijven gering in verhouding tot de fysiologische veranderingen die hierin optreden.

In **hoofdstuk 4** wordt een eenvoudig model van bloeddrukvariatie ontwikkeld dat gebaseerd is op de belangrijkste vormkarakteristiek van het 24-uurs bloeddrukpatroon, namelijk de nachtelijke bloeddrukdaling. Dit model wordt geëvalueerd aan de hand van continue intra-arteriële bloeddrukregistraties van personen met onbehandelde matige hypertensie. Tijdens de registratie verbleven deze patienten in het ziekenhuis.
We modelleerden de 24-uurs bloeddruk als een blokgolf: twee aanééngesloten perioden van constante druk die samen 24 uur lang zijn. De overgang tussen deze twee niveaus wordt voor ieder bloeddrukprofiel afzonderlijk bepaald (segmentatie) volgens een kleinste-kwadratische-fout methode. De resultaten worden vergeleken met gangbare methoden, in het bijzonder met een methode die is gebaseerd op harmonische analyse.
Segmentatie door middel van de blokgolfmethode modelleert een groter deel van de dagelijkse bloeddrukvariantie (66%) dan door middel van het oog (59%, aanzienlijke observer bias) of aan de hand van vaste tijden (50%). De "Minnesota Cosinor methode" geeft de minst goede beschrijving (47%). Bij de segmentatie gebaseerd op harmonische analyse (61%) zijn veel harmonischen nodig waardoor de segmentatie een moeizaam proces wordt, en bovendien is de betekenis van de extra informatie die wordt verkregen dubieus. Segmentatie door middel van het oog gaat mank aan observer bias en is daardoor niet geschikt is om de dagelijkse bloeddrukvariatie te kwantificeren. Zelfs als de activiteiten van de studiegroep gesynchroniseerd zijn zoals bij ziekenhuisopname, blijkt dat de tijdsonafhankelijke methoden van segmentatie de 24-uurs variantie aanzienlijk beter modelleren dan de op tijd gebaseerde methode. In vergelijking met harmonische analyse is de blokgolfmethode eenvoudig, en modelleert deze de variantie beter.

Hoofdstuk 5 becommentarieert de "periodogram-methode" die is geïntroduceerd om de 24-uurs bloeddrukvariatie te modelleren. Op grond van toepassing van dit model concluderen de auteurs dat de bloeddruk overdag bimodaal is verdeeld (twee toppen heeft). We stellen dat deze conclusie niet getrokken kan worden, omdat de nachtelijke bloeddrukdaling deze twee-toppigheid als een artefact in het toegepaste model induceert.

De precisie en juistheid waarmee van de blokgolfparameters van een 24-uurs bloeddruk-registratie kunnen worden bepaald hangt af van het aantal meetpunten in de registratie en daarmee van de meetfrequentie. De kwaliteit van de meting hangt verder af van de verhouding tussen de grootte van de nachtelijke bloeddrukdaling en de bloeddrukvariatie uit andere bron (ruis), zoals de korte-termijns bloeddrukvariabiliteit en toevallige meetfouten. Dit is met name van belang bij niet-invasieve bloeddrukmeting, een techniek met een minder grote meetfrequentie en precisie dan continue intra-arteriële meting. De genoemde afhankelijkheid wordt onderzocht in **hoofdstuk 6**. Door middel van

zogenaamde Monte Carlo experimenten wordt met behulp van gesimuleerde bloeddrukprofielen bepaald hoe de amplitude en de tijdstippen van overgang van het ene drukniveau naar het andere afhangen van de meetfrequentie, de verhouding tussen de de nachtelijke bloeddrukdaling en de standaarddeviatie van de ruis, en de lengtes van de periodes van hoge en lage druk.
Als de nachtelijke bloeddrukdaling tenminste 1.5 maal groter is dan de standaarddeviatie van de ruis, hetgeen bij de meeste gezonden het geval is, is een meetfrequentie van 2.5 per uur voldoende om de blokgolf-amplitude betrouwbaar te meten. Als de nachtelijke bloeddrukdaling gelijk is aan de standaarddeviatie van de ruis, moet tenminste 6 maal per uur worden gemeten. Als aan deze voorwaarden wordt voldaan, wordt de amplitude met niet meer dan 0.5 mmHg overschat, en neemt de standaarddeviatie van de meetfout met ten hoogste 0.6 mmHg toe. Als de nachtelijke bloeddrukdaling kleiner is dan de standaarddeviatie van de ruis wordt de methode onbruikbaar om de amplitude te bepalen omdat deze te sterk wordt overschat. Of de nachtelijke bloeddrukdaling in een verzameling registraties voldoende groot is om de blokgolfmethode te gebruiken kan worden bepaald aan de hand van de fractie significante fits.
De overgangstijden tussen de periode van hoge en lage druk zijn in mindere mate afhankelijk van de grootte van de nachtelijke bloeddrukdaling en de andere genoemde factoren. In sommige gevallen kan daarom aan de hand van de overgangstijden informatie over de fase van de bloeddrukdaling worden verkregen, ook als de amplitude niet meer berekend kan worden.

In **hoofdstuk 7** wordt het blokgolfmodel toegepast op 24-uurs bloeddrukmetingen van normotensieve, polikliniscbe personen. Deze niet-invasieve metingen verschillen van intra-arteriële registraties door een geringer aantal metingen, en grotere bloeddrukvariaties tussen de metingen. Het blokgolfmodel blijkt de 24-uurs bloeddrukschommeling goed te modelleren, en de overgangstijden van het blokgolfmodel en het begin en einde van de nachtrust volgens door de personen bijgehouden dagboekjes komen goed overeen. Deze resultaten worden verkregen na een aanpassing van de methode, namelijk een beperking van de lengte van de periode van lage druk tot ten minste vijf, en ten hoogste 13 uur. Dit bereik werd eerder met intra-arteriële metingen vastgesteld. Uit de dagboekjes blijkt verder dat het slaap-waak patroon in de groep sterk uiteenloopt. Op grond hiervan veronderstellen we dat de blokgolfmethode een betere methode lijkt om de circadiane bloeddrukvariatie in deze groep te kwantificeren dan tijdsafhankelijke methoden, een veronderstelling die door een recente studie van anderen is bevestigd. Verder blijkt dat significantie van de blokgolf-fit in deze registraties ongeveer overeenkomt met een nachtelijke bloeddrukdaling van 10 mmHg of 10%, ofwel met een "dipping profile".

Hoofdstuk 8 demonstreert de beperkingen van het blokgolfmodel aan de hand van patiënten na niertransplantatie bij wie de immunosuppressieve medicatie van cyclosporine in azathioprine wordt veranderd. In een vorige studie bij dezelfde patiënten is aangetoond dat tijdens het gebruik van cyclosporine de nachtelijke bloeddrukdaling zwakker is dan tijdens het gebruik van azathioprine. Onder beide immunosuppressieve therapieën was de diurnale bloeddrukvariatie te zwak om met het blokgolfmodel te kwantificeren. Het blokgolfmodel is dan ook niet in staat om enig verschil in amplitude tussen de twee

tussen de twee groepen aan te tonen. Wel werd een verschil gevonden tussen de groepen in de verdeling van de tijd van overgang van de periode van lage naar hoge druk.

In de hoofdstukken **9**, **10** en **11** wordt door middel van arteriële ambulante bloeddrukmeting de hemodynamiek onderzocht van personen die één tot drie jaar eerder een harttransplantatie ondergingen. Slagvolume, hartminuutvolume, en de totale perifere vaatweerstand worden geschat met een voor bloeddruk en hartfrequentie gecorrigeerde "pulse contour" methode. We vinden in deze groep een verhoogde bloeddruk en hartfrequentie. De verhoogde bloeddruk wordt waarschijnlijk veroorzaakt door het gebruik van cyclosporine. Dit immunosuppressivum induceert vochtretentie en een verhoogde sympathische activiteit, en hypertensie is een bekende bijwerking. De verhoogde hartfrequentie kan waarschijnlijk worden toegeschreven aan onderbreking van de cardiale autonome innervatie, met name aan een verminderde vagustonus.
De circadiane variatie van de hartfrequentie, berekend met behulp van een vaste dag- en nachtperiode, is in deze groep normaal, terwijl de nachtelijke bloeddrukdaling verminderd is. Dit verminderde dag-nacht verschil in bloeddruk kan voor een klein deel worden toegeschreven aan drie kortdurende bloeddrukdalingen overdag, die waarschijnlijk worden veroorzaakt door de maaltijden. In enkele registraties blijkt het dag-nacht ritme van de bloeddruk te zijn omgekeerd. De hemodynamische basis voor de verminderde nachtelijke bloeddrukdaling is een verminderde nachtelijke daling van het hartminuutminuutvolume. Een mogelijke verklaring voor deze in verhouding te grote cardiac output gedurende de nacht is de toename van de veneuze terugstroom naar het hart tijdens het liggen 's nachts. Deze wordt in het getransplanteerde hart niet gecorrigeerd door een verminderde inotropie en chronotropie. Een beperking van de sympatische modulatie van de vaatweerstand door de onderbroken ventriculo-baroreceptorreflex na harttransplantatie kan verder bijdragen aan de discrepantie tussen hartminuutvolume en vaatweerstand die gedurende de nacht bestaat.
Immunosuppressie speelt zeer waarschijnlijk ook een rol bij de vermindering van de circadiane bloeddrukverandering na harttransplantatie. Aangezien cyclosporine zowel vochtretentie als toename van de activiteit van het sympatische zenuwstelsel veroorzaakt, kan het beide genoemde mechanismen versterken. De studie bij patiënten na niertransplantatie laat zien dat cyclosporine de nachtelijke bloeddrukdaling kan verminderen ook zonder dat het hart gedenerveerd is. De mogelijke rol van cyclosporine wordt benadrukt door studies waarin de circadiane bloeddrukdaling zich enige tijd na harttransplantatie herstelt, aangezien de cyclosporinedosering in deze studies lager lag dan in de onze. Het risico op cardiovasculair lijden na harttransplantatie is verhoogd, wellicht metde als gevolg van de nog gemiddelde hogere 24-uurs bloeddrukbelasting. Een farmacotherapeutische strategie gericht op vermindering van de cyclosporinedosering en vermindering van de veneuze terugstroom naar het hart gedurende de nacht is daarom mogelijk van waarde bij het verbeteren van de prognose van deze patiënten op lange termijn.

Alles samenvattend hebben we een eenvoudige techniek geïntroduceerd die de circadiane bloeddrukvariatie modelleert door het 24-uurs bloeddrukprofiel in een periode van hoge en een periode van lage druk te verdelen. Deze techniek kan aan- en afwezigheid van een circadiaan ritme detecteren, en de amplitude en de overgangstijden tussen beide perioden

kwantificeren. Als het circadiane ritme is verzwakt, kan de methode de amplitude niet meer bepalen, maar vaak nog wel informatie over de fase van het ritme verschaffen.

Mogelijke vragen voor verdere studie zijn:

- Kan de amplitude van de nachtelijke bloeddrukdaling worden berekend door het bloeddrukprofiel in een dag- en een nacht-periode te verdelen, waarbij de overgangstijden door de blokgolftechniek zijn berekend uit het overeenkomstige hartfrequentie-profiel? Deze vraagstelling is met name van belang indien de nachtelijke bloeddrukdaling te gering is om met behulp van de blokgolf-methode rechtstreeks uit het 24-uurs bloeddrukprofiel te berekenen.
- Kan de robuustheid van de blokgolf-methode worden verbeterd door een optimalisatietechniek die niet is gebaseerd op de kruiscorrelatiecoëfficiënt? Dit is van belang omdat hierdoor het belang van het verwijderen van sterk afwijkende meetwaarden (uitbijters) uit de registraties vermindert.
- De belangrijkste vraag betreft de klinische relevantie van de blokgolf-maat van circadiane bloeddrukvariatie met betrekking tot morbiditeit, mortaliteit en orgaanschade, en hoe deze zich verhoudt tot de relevantie van de verschillende andere maten van circadiane bloeddrukvariabiliteit. Bij het beantwoorden van deze vraag kan gebruik worden gemaakt van gegevens uit gepubliceerd onderzoek.

Curriculum vitae

Naam	Reijerus (René) Nicolaas Idema
Geboortedatum	17 september 1959
Burgerlijke staat	Gehuwd met K. Baurichter, huisarts. Kinderen Matthijs (1994) en Merith (1995)

Opleiding

- VWO-ß, Praedinius Gymnasium te Groningen (1971-1977)
- Scheikunde, Rijksuniversiteit Groningen (1977-1978)
- Geneeskunde, Rijksuniversiteit Groningen (1978-1986)
- Postdoctorale opleiding Medisch Informatica, Prof. Dr. Ir. J.H. van Bemmel, Vrije Universiteit Amsterdam, Erasmus Universiteit Rotterdam (1986-1988)
- Specialisatie tot Arts Klinische Chemie, Dr. E.W. Kuijpers, Sophia Ziekenhuis Zwolle (1992-1996)
- Stralingsdeskundige Niveau 3A, Rijksuniversiteit Groningen (1995)

Aanstellingen tot op heden

- Student-assistent Klinische Farmacologie, Rijksuniversiteit Groningen, onderwijs en onderwijsontwikkeling (1983)
- Student-assistent Onderwijsontwikkeling, Rijksuniversiteit Groningen, ontwikkeling van programmatuur voor computer-ondersteund onderwijs (1984)
- Onderzoeksmedewerker bij de vakgroepen Inwendige Geneeskunde I, Dijkzigt Ziekenhuis Rotterdam, en Medische Informatica, Erasmus Universiteit Rotterdam (1988-1992)
- Arts-assistent in opleiding tot Arts Klinische Chemie, Sophia Ziekenhuis Zwolle (1992-1996)
- Arts Klinische Chemie, Samenwerkingsverband Klinisch-chemische Laboratoria van het Holy Ziekenhuis te Vlaardingen en het Schieland Ziekenhuis te Schiedam

Overige

1986 Free-lance ontwikkeling programmatuur voor onderwijsevaluatie

Publicaties

Idema RN, van den Meiracker AH, Imholz BPM, Man in 't Veld AJ, Settels JJ, Schalekamp MADH: Comparison of Finapres non-invasive beat-to-beat finger blood pressure with intra-brachial artery pressure during and after bicycle ergometry. J Hypertens 1989;7(6):S58-S59

Idema RN, Gelsema ES, Wenting GJ, Grashuis JL, van den Meiracker AH, Brouwer ML, Man in 't Veld AJ: Square wave fitting: a new method of characterizing diurnal variation in blood pressure. J Hypertens 1991;9(6):S326-S327

Idema RN, Gelsema ES, Wenting GJ, Man in 't Veld AJ: Modeling blood pressure profiles. Hypertension 1992;19: 496-497 (letter)

Idema RN, Gelsema ES, Wenting GJ, Grashuis JL, van den Meiracker AH, Brouwer ML, Man in 't Veld AJ: A new method for diurnal blood pressure profiling - square wave fit compared with conventional methods. Hypertension 1992;19:595-605

Idema RN, van den Meiracker AH, Man in 't Veld AJ, Balk AHMM, Bos E, Schalekamp MADH: Circadian hemodynamics in heart transplant recipients. J Hypertens 1993;11(5): S90-S91

Idema RN, van den Meiracker AH, Balk AHMM, Bos E, Schalekamp MADH, Man in 't Veld AJ: Decreased circadian blood pressure variation up to three years after heart transplantation. Am J Cardiol 1994;73:1006-1009

Idema RN, van den Meiracker AH, Balk AHMM, Bos E, Schalekamp MADH, Man in 't Veld AJ: Abnormal diurnal variation of blood pressure, cardiac output, and vascular resistance in cardiac transplant recipients. Circulation 1994;90:2797-2803

Treskes M, Adriaansen HJ, van de Leur JJCM, Idema RN, Péquériaux N, Pronk C: Dutch multi-center evaluation of the EBIO Plus glucose analyser. Accepted for publication in the Eur J Clin Chem Clin Biochem

Jaarsma AS, Baerts W, Idema RN, Samson G, Fetter WPF, Bos AP: Pro-ANP levels during high frequency oscillatory ventilation in preterm infants (submitted)

Idema RN, van den Meiracker AH, Man in 't Veld AJ, Gelsema ES: Dependency of square wave parameters on measurement frequency, noise, and diurnal variation of blood pressure (submitted)

Diamant M, Idema RN, Vincent HH: The use of Fourier analysis in the calculation of trough-to-peak ratio from ambulatory blood pressure measurements (submitted)

Hartgerink M, Posma FD, Hessels J, Idema RN, Wolthuis J: Decreased circadian variation of TSH in the elderly (in preparation)

Abstracts en posters

Idema RN, van den Meiracker AH, Imholz BPM, Man in't Veld AJ, Settels JJ, Schalekamp MADH: Comparison of Finapres non-invasive beat-to-beat finger blood pressure with intra-brachial artery pressure during and after bicycle ergometry. Milan: Universita degli Studi di Milano, Ricerca Scientifica ed Educazione Permanente 1989, S76:378; abstract

Idema RN, van den Meiracker AH, Man in 't Veld AJ, Wenting GJ, Brouwer ML, Schalekamp MADH: Chaos-, grey scale- and iso-frequency contour plots. A new method for visual integration of heart rate and blood pressure applied to continuous 24-hour blood pressure measurement. J Hypertens 1990;8(3):S87; abstract

Idema RN, Brouwer ML, Grashuis JG, Gelsema ES, Wenting GJ, Schalekamp MADH: The square wave fitting method. A new method to characterize circadian blood pressure and heart rate variability compared to other methods. Milan: Universita degli Studi di Milano, Ricerca Scientifica ed Educazione Permanente 1991, S 96:254; abstract

Idema RN, van den Meiracker AH, Man in 't Veld AJ, Balk AM, Schalekamp MADH: Reduced circadian blood pressure variation in heart transplant recipients is caused by high cardiac output and vascular resistance at night. Milan: Universita degli Studi di Milano, Ricerca Scientifica ed Educazione Permanente 1993, S 104:213; abstract

Wenting GJ, Brouwer ML, Idema RN, Schalekamp MADH: Diurnal blood pressure load and control in cyclosporine treated hypertensive renal transplant recipients. In: Proceedings of the Fifth International Symposium on Calcium Antagonists: Pharmacology and Clinical Research. 1991, Houston, Texas, USA; posterpresentatie

Hartgerink M, Idema RN, Hessels J, Posma FD: 24-hour TSH and fT4 pattern in elderly and young adults: Implications for blood sampling and reference values. IFCC, 11th Eur Symp Clin Chem, July 1995, Helsinki, Finland; posterpresentatie

Jaarsma A, Bos AP, Baerds W, Idema RN, Samson G: Atrial natriuretic peptide (ANP) during high frequency oscillatory ventilation (HFVO) in preterm infants. Annual Meeting of the European Society of Pediatric Research, september 1995, Alicante, Spanje; posterpresentatie

Treskes M, Adriaansen HJ, van de Leur JJCM, Idema RN, Péquériaux N, Pronk C: Multicenter evaluatie van de EBIO Plus glucose analyser. Achtenveertigste NVKC congres, 11 april 1996, Lunteren; posterpresentatie

Dankwoord

Gedurende het overgrote deel van de periode waarin een proefschrift tot stand komt is de blik van de onderzoeker vooruit gericht. Het is pas na de uitdrijvingsfase en vlak vóór het doorknippen van de navelstreng dat de onderzoeker in verbijstering neerziet op de boreling en zich realiseert dat dit succesje weliswaar één moeder, maar ook vele vaders heeft. De gedachten gaan terug langs het lange traject dat vanaf de conceptie doorlopen is en de onderzoeker begint te beseffen wat er eigenlijk allemaal gebeurd is. Deze opkropping verklaart dat hij zijn meestal gortdroge boekwerkje afsluit met een finale van emoties en persoonlijke gedachten, hetgeen voor de niet-ingewijde mogelijk enigszins bevreemdend werkt. Dit gezegd hebbend, kan ik verder vrijuit spreken.

Mijn dank gaat uit naar mijn promotor, professor dr. E.S. Gelsema. Edzard, jij hebt me geleerd bevindingen te ordenen en te verwoorden. Het ontwikkelen van deze kunde is essentieel geweest voor het op gang komen van het onderzoek. Door mee te denken op de momenten dat het nodig was en me op de andere momenten vrijheid te laten heb je een klimaat geschapen waarin het goed werken was. Je rustige aanwezigheid en permanente beschikbaarheid waren de stabiele achtergrond waartegen ik me kon aftekenen. De enige wanklanken waren te beluisteren tijdens de wekelijkse koorrepetities.

Net zozeer gaat mijn dank uit naar mijn promotor professor dr. A.J. Man in 't Veld. Arie, door je nooit-aflatende energie, ideeën en daadkracht ben je net zozeer "motor" als "promotor" geweest. Je steun, vertrouwen, je rusteloos verder zoeken en je inzicht in de (ir)relevantie van bevindingen waren onmisbaar voor dit onderzoek. Met genoegen herinner ik me een recente zomeravond waarop we hoog boven de Kralingse grond zweefden.

Dr. A.H. van den Meiracker, Ton, niemand kan dagelijkse beslommeringen zo verwoorden als jij. Ik prijs me gelukkig dat mijn onderzoek nogal eens tot die beslommeringen behoorde. Met gedegenheid heb je de fundamenten onder een belangrijk deel van de studies gemetseld. Je bent mijn drijfanker geweest in de tijd dat ik in Zwolle in opleiding was, en hebt niet geaarzeld soms de rol van sleepboot op je te nemen. Ik dank je voor je vriendschap.

Dr. G.J. Wenting, Gert-Jan, veel van wat voor voornoemde personen geldt, geldt a fortiori voor jou. Je hebt me in alle opzichten en onvoorwaardelijk gesteund in onderzoek en werk. Bovenal heb je het voor me mogelijk gemaakt om verdere stappen te nemen. Ik ben je hiervoor bijzonder erkentelijk.

Dr. Ir. J.L. Grashuis, beste Jan, je bent voor mij in de eerste plaats een van de meest markante en gastvrije gezichten van Rotterdam en van de vakgroep Medische Informatica geweest. Daarnaast heb je voor de noodzakelijke organisatorische en mathematische basis voor dit onderzoek gezorgd. Voor beide dank ik je.

Professor dr. ir. J.H. van Bemmel, Jan, dankzij jou heb ik naast mijn medische een exacte achtergrond verworven terwijl dat eigenlijk helemaal niet mogelijk was. Ik dank je

hiervoor, voor de gastvrijheid op de vakgroep Medische Informatica, voor je steun bij het onderzoek en voor je bereidheid het voorliggende werk op zijn wetenschappelijke waarde te beoordelen.
Professor dr. M.A.D.H. Schalekamp, Maarten, ik denk met genoegen terug aan het persoonlijk contact tijdens congressen of informele bijeenkomsten van Inwendige Geneeskunde I. Ik dank je voor de gastvrijheid op deze afdeling en voor je bereidheid dit werk te beoordelen.
Dr. Ir. A.L.D. Beckers, beste Guus, vaak zijn we elkaars klankbord geweest voor doorgemaakt wetenschappelijk, niet-wetenschappelijk en pseudo-wetenschappelijk lief en leed. Je hebt de kiem gelegd van mijn inzicht in werkelijk belangrijke datastructuren. Als kamergenoot en collega heb je meer voor me betekend dan ik in hier kan zeggen, en ik ben daarom blij dat jij mijn paranimf wilt zijn.
Dr. Ir. T.D. Meijler, beste Theo Dirk, net als Guus was je mijn klankbord en natuurlijk *sparring partner* voor woordgrapjes, waarbij die van jouw net iets frequenter en spitser waren dan die van mij. Ik dank je voor je vriendschap.
Prof. Dr. Ir. A. Smeulders, Arnold, mede dankzij jouw hulp en flexibiliteit heb ik een voor mij belangrijk doel kunnen bereiken. Tijdens de beginfase van het onderzoek was je degene die terloops, in de kortste tijd, met de minste woorden, en puttend uit je kennis op vele terreinen, een maximum aan relevante informatie kon overdragen, en dit ook daadwerkelijk deed. Zelf zou je dit verwoorden als "optimale communicatie".
Drs. M.L. Brouwer, beste René, hoewel onze wegen uiteenliepen bewaar ik aan onze samenwerking goede herinneringen.
Ik dank de medewerkers van de Centrale Instrumentele Dienst J. Smallegange, A.P.A. Noordermeer en R. Visser. Joop, Fons, en Bob, het was me een waar genoegen met een enthousiast team als dat van jullie te werken.
Ing. P.A. Kruysse, Piet, jou dank ik voor je hulp bij het ontwikkelen van een gebruikersinterface voor de analyseprogrammatuur.
Dr. Ir. J.E. Vos, Hans, je hebt me acht jaar geleden precies de helft heeft gegeven van een idee waardoor het huidige onderzoek zijn kritische impuls kreeg. Ik dank je hiervoor.
Ik dank alle medewerkers van de vakgroep Medische Informatica, waar ik het meest vertoefde, en van Inwendige Geneeskunde I, voor de collegialiteit, de discussies, en de gezelligheid.

In het bijzonder wil ik nogmaals diegenen onder de voornoemden danken door wier raad, vasthoudendheid en daden dit werk afgerond kon worden. Mede aan hun dank ik dat ik met voldoening op de afgeronde periode kan terugzien, en naar de toekomst uitzie.
Tot slot bedank ik mijn ouders voor hun liefde, hun zorg, en de vrijheid die ik gehad heb, zonder welke dit alles niet mogelijk zou zijn geweest.
Kristine, jouw begrip, je liefde, en je voortdurende steun zijn de redenen waarom ik dit werk aan jou opdraag.

Zwolle, 12 september 1996