Variation in non-invasive measurements of vascular function in healthy volunteers during daytime

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ABSTRACT

Although it is often recommended to standardize the time of day when performing non-invasive measurements of vascular function, the exact influence of the time of day on the outcome of IMT (intima-media thickness), PWV (pulse wave velocity), AIX (augmentation index) and FMD (flow-mediated dilatation) measurements has not been reported before. Nineteen healthy volunteers visited our department on two different occasions: the first visit was at 09:00 hours after an overnight fast, and the second visit was at 14:00 hours after a standardized breakfast. Non-invasive measurements of atherosclerosis were performed twice at 09:00 hours and once on the second visit at 14:00 hours. Measurement of IMT, PWV, AIX and FMD was reproducible according to the method of Bland and Altman. The absolute difference between repeated measurements at 09:00 hours showed no significant difference compared with the absolute difference between 09:00 and 14:00 hours for IMT (0.029 ± 0.014 compared with 0.021 ± 0.014 mm; P = 0.27), PWV (0.63 ± 0.50 compared with 0.75 ± 0.74 m/s; P = 0.52), AIX (4.0 ± 4.0 compared with 5.5 ± 5.2%; P = 0.35) and FMD (3.8 ± 3.7 compared with 4.2 ± 2.9%; P = 0.70). In conclusion, our results show that, in healthy volunteers during the daytime, IMT, PWV, AIX and FMD outcomes are not confounded by variation in the exact time of the examination as long as other (exogenous) conditions, including food intake, smoking and intake of alcohol, are carefully controlled for.

INTRODUCTION

Atherosclerosis is known to be a gradual process which can be measured with a variety of non-invasive techniques, each of which quantifies a different stage in the atherosclerotic process. Early functional changes can be quantified by FMD (flow-mediated dilatation), AIX (augmentation index) and PWV (pulse wave velocity), whereas IMT (intima-media thickness) and ABI (ankle-brachial blood pressure index) measurements can be used to quantify later and more structural changes in the arterial wall. As NIMA (non-invasive measurements of atherosclerosis) become more and more important in clinical practice to evaluate the risk of CHD (coronary heart disease) and the effect of treatment, reliable and reproducible tests should be available, preferably during the course of the whole day for practical reasons.

It is recommended by International Task forces [1,2] to standardize patient conditions when performing NIMA, since there is abundant evidence that various exogenous variables, including smoking [3,4], vitamin intake [5,6], alcohol intake [7,8], use of oral contraceptives [9] and food intake [10–12], affect the outcome of PWV, AIX and FMD measurements. It is also often recommended

Key words: augmentation index, diurnal variation, flow-mediated dilatation, intima-media thickness, pulse wave velocity.
Abbreviations: ABI, ankle-brachial blood pressure index; AIX, augmentation index; CHD, coronary heart disease; FMD, flow-mediated dilatation; IMT, intima-media thickness; NIMA, non-invasive measurements of atherosclerosis; PWV, pulse wave velocity.
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to standardize the time of NIMA. However, data about the effect of the time of day of the measurements on AIX, PWV and IMT outcomes are lacking. Only a few studies have addressed the effect of the time of measurement on FMD outcomes, with opposing results [13–15]. In these studies, the ‘overall circadian variation’ in FMD, due to both variation in the point of time of the measurement and to variation in exogenous influences, such as physical activity and food intake, was investigated. Hence recommendations to standardize the time of measurements are thus confounded by postprandial haemodynamic changes, since food intake exerts a significant effect on cardiac output, heart rate [16] and blood pressure [17], all determinants with an important influence on NIMA.

The aim of the present study was to quantify the effect of the time of day on the outcome of four NIMA, including IMT, PWV, AIX and FMD.

METHODS

Subjects
A total of 19 healthy volunteers, aged 25–63 years, without a history of cardiovascular diseases, hypertension or diabetes, were studied. None of the participants used any medication or vitamin preparations, except for one subject who used atorvastatin. The Medical Ethics Committee of the UMC Nijmegen approved the study protocol, and all participants provided written informed consent.

Experimental protocol
Subjects were invited to come to the vascular laboratory twice within 2 weeks. The first visit was at 09:00 hours after an overnight fast, and the second visit at 14:00 hours after a normal breakfast of 400 kcal (where 1 kcal = 4.184 kJ) at 08:00 hours. On both occasions the subjects abstained from alcohol and caffeine-containing products for at least 24 h, and did not smoke for at least 6 h before the measurements.

At the 09:00 hours visit, AIX, PWV and IMT measurements were carried out twice by two sonographers each within 30 min in order to estimate reproducibility of these techniques. Therefore, in total, four measurements were performed for each patient. FMD was measured only once by one sonographer, since reproducibility for this entity has already been obtained in a preliminary study, including 15 healthy volunteers, aged 23–56 years, with FMD measured twice after an overnight fast on two different morning occasions within 1 week (results reported in the present study).

Single measurements of IMT, PWV, AIX and FMD were repeated by one sonographer at the 14:00 hours visit in order to be able to quantify the effect of measurement time as a cause of variability in outcome.

Laboratory measurements
On both occasions, peripheral blood was drawn. The plasma glucose was measured using the glucose oxidation method (Glucose Analyser II; Beckman, Palo Alto, CA, U.S.A.). Plasma triacylglycerol (triglyceride) concentrations were determined by a commercially available enzymatic reagent (Sera Pak; Miles, Italy).

FMD measurements
Ultrasound images of the brachial artery were obtained using a 7.5 MHz linear-array transducer of an AU5 ultrasound system (Esaote Biomedica, Genova, Italy) connected to a computer with a data acquisition board. Dedicated software (Wall Track System 2.0; Pie Medical, Maastricht, The Netherlands) was used to measure and analyse the changes in vessel diameter. All measurements were performed by the same trained sonographer and analysed off-line independently by another investigator.

FMD was assessed in the subject’s right arm in the supine position in a temperature-controlled room (24 °C) after a 10-min equilibration period. For image optimization, we used a stereostatic arm with a holder for the probe and a gel pad to optimize focal depth and prevent compression of the artery. Measurement of FMD was by the method of Celermajer et al. [18]. Briefly, three baseline measurements of brachial artery diameter and flow velocity were performed, after which a pneumatic tourniquet (placed around the forearm distal to the segment of artery scanned) was inflated. After 4 min, the tourniquet was deflated and flow velocity was recorded instantly. Vessel diameter was recorded continuously in 4-s frames every 15 s after deflation for at least 4 min to detect shear-stress-mediated changes in vessel diameter. FMD was calculated as the difference between the maximum post-occlusive diameter and the average baseline diameter, relative to the average baseline diameter, and expressed as a percentage.

AIX and PWV measurements
AIX was determined by recording the peripheral pulse wave on the radial artery by the Sphygmocon system (Atcor Medical, Sydney, Australia) using a high-fidelity micromanometer (SPC-301; Millar Instruments, Houston, TX, U.S.A.) after the subject had had a 10-min supine rest. After 20 sequential pulse waveforms had been recorded, an average peripheral waveform was generated, from which ejection duration was determined. Subsequently, the corresponding central waveform was derived by applying a validated integral transfer function on the average peripheral waveform, after which AIX was calculated [19–21]. All waveforms were calibrated using the brachial blood pressure, which was recorded in accordance with international recommendations [22], immediately before AIX measurement using an oscillometric sphygmomanometer (Critikon model no. 1846,
Critikon, Tampa, FL, U.S.A.). Since AIX is inversely correlated to the subject’s heart rate [23], correction for this parameter was carried out for each individual.

To determine PWV, pulse waveforms were recorded at two sites (right carotid artery and left femoral artery), and wave transit-time was calculated using the R-wave of a simultaneously recorded ECG as a reference frame. Then surface distance between the two recording sites was measured in order to calculate PWV. To reduce the influence of body contours on the distance measure, the tape measure was held above the surface of the body parallel to the plane of the examination table. All measurements of AIX and PWV met the criteria of optimal quality as defined by the manufacturer.

**IMT measurements**

Carotid IMT was determined using an AU5 Ultrasound machine (Esaote Biomedica) with a 7.5 MHz linear-array transducer. Longitudinal images of the most distal 10 mm of both the far wall and the near wall of both common carotid arteries were obtained in the optimal projection (anterolateral, lateral or posterolateral), as described previously [24]. The same projection was used for the two 09:00 hours scans as well as the 14:00 hours scan. The actual measurement of IMT was performed off-line by the sonographer at the time of the examination using semi-automatic edge-detection software (M’Ath®Std version 2.0, Metris, Argenteuil, France), as described previously [25]. All measurements were carried out in end-diastole again using the R-wave of a simultaneously recorded ECG as a reference frame. From each frame the mean IMT was calculated over at least 7.5 mm of the above-mentioned 10 mm segment (yielding a quality index of at least 75%). The outcome variable was defined as the mean IMT of the near and far wall of both common carotid arteries.

**Statistical analysis**

Values are given as means ± S.D., unless otherwise stated. As the normal physiological range for AIX is from negative to positive values, the use of the coefficient of variance is inappropriate. Instead, all data were analysed using Bland–Altman plots, and reproducibility was expressed in terms of both absolute differences and mean difference ± S.D. between paired measurements for all variables, as described by Bland and Altman [26].

The Shapiro–Wilk algorithm was used to determine whether each variable had a normal distribution. Paired (IMT, PWV and AIX) and unpaired (FMD) two-tailed Student t tests or Wilcoxon signed-rank tests were used to test for significant differences where appropriate. A P value < 0.05 was considered as significant. Statistical analyses were performed using SPSS 12.0.1 for windows (SPSS, Chicago IL, U.S.A.) and Excel 2000 (Microsoft, Seattle, WA, U.S.A.).

**RESULTS**

**Subject characteristics**

The study population consisted of five male and 14 female subjects. Eleven of the female subjects were pre-menopausal, and five of them were using oral contraceptives. The mean age of the subjects was 39.3 ± 10.5 years. They had a mean BMI (body mass index) of 22.9 ± 2.4 kg/m², and a mean WHR (waist/hip ratio) of 0.82 ± 0.07. There were three current smokers, and the mean alcohol intake was 5.6 international units/week.

No significant differences in the mean values of systolic and diastolic blood pressure, heart rate, ejection duration and triacylglycerol and glucose levels were observed between 09:00 and 14:00 hours (Table 1).

**Repeatability of NIMA**

There was no significant difference between the NIMA in the two recordings. The absolute difference, averaged for both investigators, for repeated measurements at 09:00 hours was 0.029 ± 0.014 mm for IMT, 4.0 ± 4.0 % for AIX and 0.63 ± 0.50 m/s for PWV. For FMD, the absolute difference between repeated measurements was 3.8 ± 3.7 %. Bland–Altman plots for the mean within-observer variability in measurements of both observers are shown in Figure 1, and do not show any trend for reproducibility being dependent on the underlying mean value. The mean ± S.E.M. difference between the first and second recordings at 09:00 hours was 0.003 ± 0.007 mm for IMT, 0.68 ± 0.86 % for AIX, 0.19 ± 0.12 m/s for PWV and −1.05 ± 1.12 % for FMD. The S.D. of the mean differences was 0.032 mm for IMT, 4.87 % for AIX, 0.52 m/s for PWV and 4.87 % for FMD. Since (more than) 95 % of all readings for IMT, AIX, PWV and FMD were within the error range defined by the coefficient of repeatability, we conclude that the procedure is reproducible as defined by the British Standards Institution [27].

<table>
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<th>Table 1 Subject characteristics (n = 19)</th>
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<td><strong>Variable</strong></td>
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<td>Glucose (mmol/l)</td>
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Morning–afternoon effect on the outcome of NIMA

The Bland–Altman plots for the variability in measurements between 09:00 and 14:00 hours are shown in Figure 2, and do not show any trend for variability to be dependent on the underlying mean value. The mean ± S.E.M. difference between 09:00 and 14:00 hours was 0.009 ± 0.005 mm for IMT, 0.95 ± 1.68 % for AIX, −0.37 ± 0.23 m/s for PWV and −0.71 ± 1.18 % for FMD. The S.D. of the differences between 09:00 and 14:00 hours was 0.023 mm for IMT, 7.35 % for AIX, 1.00 m/s for PWV and 5.15 % for FMD. All observations for PWV and FMD and all but one observation (95 %) for AIX and IMT lay within the error range defined by the repeatability coefficient.

Although there appeared to be a tendency towards slightly lower FMD at 09:00 hours compared with 14:00 hours (4.47 ± 4.53 % compared with 5.18 ± 3.55 %), the absolute difference was not significant. The same holds for PWV measurements (6.46 ± 1.08 compared with 6.83 ± 1.13 m/s). On the contrary, there was a tendency towards slightly higher values at 09:00 hours compared with 14:00 hours for AIX measurements (9.8 ± 14.5 compared with 8.9 ± 13.4 %), but here differences were also not significant. IMT was equal at 09:00 hours and the 14:00 hours recording (0.63 ± 0.08 mm).

Repeated measurements at 09:00 hours compared with morning–afternoon differences

The absolute difference between repeated measurements at 09:00 hours showed no significant difference compared with the absolute difference between 09:00 and 14:00 hours for IMT (0.029 ± 0.014 compared with 0.021 ± 0.014 mm; P = 0.27), PWV (0.63 ± 0.50 compared with 0.75 ± 0.74 m/s; P = 0.52), AIX (4.0 ± 4.0 compared with 5.5 ± 5.2 %, P = 0.35) and FMD (3.8 ± 3.7 compared with 4.2 ± 2.9 %; P = 0.70).

DISCUSSION

The reproducibility of the IMT, PWV, AIX and FMD measurements in the present study is comparable with studies reported previously [28–31]. However, the present study is complementary with these previous studies by showing that reproducibility remains high, even when measurements are carried out at different time points during the day, as long as all other patient conditions are carefully standardized. Furthermore, we have shown that the absolute differences between repeated measurements at 09:00 hours are equal to the absolute differences between the measurements at 09:00 and 14:00 hours.
Diurnal variation of non-invasive measurements of vascular function 429

Figure 2  Bland–Altman plots showing the differences between measurements at 09:00 and 14:00 hours against the mean value
(a) FMD; (b) AIX; (c) PWV; (d) IMT. Mean value is represented by a solid line, and 1.96 S.D. by a dotted line.

Therefore, even if there is biological variability in IMT, PWV, AIX and FMD due to variation in the time of day of the measurements, its effect is minor compared with measurement variability. This has important implications, since NIMA are accepted as reliable surrogate markers of the process of atherosclerosis and are frequently used in clinical trials to evaluate therapeutic interventions.

Our present data show that it is not mandatory to standardize the time of NIMA. This appears to be in contradiction with the observation that a close interaction between the sympathetic nervous system and the endothelium exists [32–34]. Diurnal changes in sympathetic activity are generally held responsible for diurnal changes in NO (nitric oxide) availability and a morning surge in cardiovascular events.

However, in our present study, we did not find sympathetic activity (and resultant NO availability) at 09:00 hours to be different from 14:00 hours; there were no differences in heart rate and other cardiovascular physiological parameters (systolic blood pressure/diastolic blood pressure and ejection duration) between recordings at 09:00 and 14:00 hours, whereas Hijmering et al. [33] showed that heart rate and (muscle) sympathetic nerve activation are closely related.

This could simply be explained by the fact that all recordings at 09:00 hours were performed a substantial period of time after the patient woke up. Therefore, by the time of measurement, the characteristic morning surge in cortisol and catecholamine blood levels and the resultant concomitant change in related cardiovascular parameters (such as blood pressure, heart rate and total peripheral resistance) had already taken place [35].

Previous studies have addressed the topic of circadian variation in FMD [13–15]. In contrast with the present study, they did find clues for the existence of a circadian rhythm. Some groups found significantly higher FMD in the afternoon compared with the morning [13,15], whereas others found the contrary [14]. The discrepancy with our present findings could be explained by the fact that the studies mentioned above investigated the composite effect on endothelial function of both endogenous factors (such as circadian autonomic nerve activity) and certain exogenous factors (such as food intake, smoking and caffeine intake), whereas, in our present study, these conditions were carefully controlled for and, as a consequence, plasma glucose and triacylglycerol levels were equal at 09:00 and 14:00 hours.

Since different studies showed that FMD is remarkably affected shortly after food intake and that these changes last for 4–6 h [11,12], we may conclude that exogenous conditions (such as food intake) probably exert an influence on FMD measurements which is more extensive than the influence of variability in the exact time of the measurements.
Variability in food intake during the day is also a reason for the Task Force III on Clinical Applications of Arterial Stiffness advising measurements of arterial stiffness (such as PWV and AIX) to always be performed at the same time of day [2]. It is common knowledge that food intake decreases systemic vascular resistance, with an accompanied increase in heart rate and cardiac output and, particularly in elderly subjects, a decrease in blood pressure [17].

Since a direct correlation exists between (diastolic) blood pressure and both AIX [36] and PWV [37], as well as an inverse correlation between heart rate and AIX [38], we may assume that measurements of vascular stiffness are significantly affected in the postprandial state.

Previous studies have shown significant differences in diameter and compliance of large arteries between nighttime and daytime [2,39], which were attributed primarily to circadian changes in autonomic activity. Our present data suggest that, during daytime, the variability in sympathetic activity is too small to demonstrate the resultant variability in stiffness properties of large arteries as measured by PWV and AIX when standardization for exogenous conditions has taken place.

Finally, we could not demonstrate any variability in IMT during the daytime. However, we did not expect to find any differences between the measurements at 09:00 and 14:00 hours, since IMT is a marker of more structural changes in the atherosclerotic vessel wall and is supposed to be insensitive to short-term alterations in endogenous and/or exogenous variables, such as sympathetic output and food intake.

In conclusion, the results of the present study have the practical implication that, when evaluating the risk of CHD or therapeutic interventions on endothelial function or vascular stiffness with NIMA, it does not appear mandatory to always perform measurements at exactly the same time of the day, as long as the other experimental conditions are carefully standardized. However, since both measurement times in the present study are during the daytime (with blunted circadian variation in sympathetic activity and the resultant heart rate and blood pressure), the results cannot be extrapolated over the whole day. Furthermore, it must be noted that the present study was performed in a group of healthy volunteers, and that the results cannot be automatically extrapolated to patients suffering from cardiovascular diseases, especially when they have disturbed lipid metabolism and/or insulin resistance. Further research is required to study the influence of time variability on measurement outcomes in these groups of patients.

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REFERENCES

Diurnal variation of non-invasive measurements of vascular function


27 British Standards Institution (1979) Precision of Test Methods, BSI, London


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