Diurnal variation and repeatability of arterial stiffness and cardiac output measurements in the third trimester of uncomplicated pregnancy

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ABSTRACT

Aim

To investigate same day repeated measures and diurnal variation of arterial stiffness, cardiac output (CO), stroke volume (SV) and total peripheral resistance (TPR) during the third trimester of normal pregnancy.

Methodology

Pulse wave velocity (PWV) and augmentation index (AIx) were recorded using the Arteriograph®, while CO, SV and TPR were recorded using NICOM®. The measurements were obtained in the third trimester of pregnancy from 21 healthy pregnant women at four time points (morning, afternoon, evening and midnight) over a 24h period. Triplicate measurements of 67 women were obtained at five-minute intervals to assess repeatability between measurements within a subject.

Results

Diurnal measurements of arterial stiffness for brachial AIx, aortic AIx and PWV were not statistically significantly different at any of the four time points. Estimated means (standard deviation) for PWV at the four stated time points were 7.81 (2.05), 8.45 (1.68), 7.87 (1.74) and 7.64 m/s (1.15), respectively, (p=0.267). Estimates for AIx at those time points were 10.22 (15.62), 4.44 (10.07), 6.49 (10.92) and 8.40% (8.16), respectively, (p=0.295). Similarly, Mean Arterial Pressure (MAP), Stroke Volume (SV), Stroke Volume Index (SVI) and Total Peripheral Resistance (TPR) did not show any evidence of diurnal variation. However, we observed that the mean Cardiac Output (CO), Cardiac Index (CI) and Heart Rate (HR) varied
from morning to midnight; the mean CO, HR and CI increased significantly in the afternoon compared with the corresponding mean morning measurements in a similar fashion to HR. Mean (standard deviation) CO estimates at the four stated time points were 5.90 (1.33), 6.38 (1.49), 6.18 (1.43) and 5.80 ml/min (1.19), respectively (p<0.001), while mean CI estimates were 3.65 (0.58), 3.93(0.68), 3.81 (0.65), and 3.57 (0.48), respectively (p<0.001) and mean HR estimates were 95 (12), 98 (13), 95 (12) and 88 (12.98), respectively (p<0.001).

Triplicate measurements of 61 women in our repeatability study showed moderate to high correlation between observations on the same woman for all Arteriograph and NICOM variables (estimates of intraclass correlation ranged from 0.49 to 0.91).

**Conclusion**

With the exception of CO, CI and HR which showed a diurnal variation, measurements of most haemodynamic parameters did not change significantly from morning to midnight suggesting there was no evidence of systematic differences in the mean values of these variables at these time points. Multiple consecutive non-invasive measurements of vascular stiffness, CO, SV and TPR were highly correlated confirming repeatability of measurements in the third trimester of uncomplicated pregnancy, so these haemodynamic measurements do not need to be undertaken at a specific time period of the day.
INTRODUCTION

The cardiovascular system undergoes positive adaptations during pregnancy. In normal pregnancy, arterial stiffness decreases during the first trimester and remains low until the end of pregnancy due to either reduced smooth muscle tone or vessel wall remodelling\textsuperscript{1,2}. Furthermore, there is an increase in intravascular volume, cardiac output (CO), and heart rate (HR), together with a decrease in vascular resistance and mean blood pressure (BP) from as early as six weeks gestation\textsuperscript{2-4}. CO steadily increases from the first trimester of pregnancy to 45% above the non-pregnant level at 24 weeks of gestation, it then plateaus but remains elevated until term\textsuperscript{2}, and is a consequence of increased HR and decreased systemic vascular resistance (SVR). From the 5\textsuperscript{th} week of pregnancy, there is a decline in SVR, which reaches a nadir between weeks 20 and 32 weeks\textsuperscript{4,5}, and is due to changes in resistance and flow in multiple vascular beds, such as the utero-placental unit.\textsuperscript{4} Thereafter, there is a gradual increase in SVR from 32 weeks until term\textsuperscript{4,5}.

Increased systemic arterial stiffness has been reported among women with hypertensive disorders during pregnancy\textsuperscript{6-8}, is associated with foetal growth restriction\textsuperscript{9}, and may have a role as a potential screening tool in pregnancy\textsuperscript{10}. Central arterial stiffening is associated with adverse cardiovascular outcomes in various patient groups\textsuperscript{11}, as well as in the general population\textsuperscript{12}; associated increased pulse wave velocity (PWV) being an independent predictor of cardiovascular morbidity and mortality\textsuperscript{13-16}. European Society of Hypertension guidelines propose that in arterial hypertension, PWV over 12m/s suggests sub-clinical organ damage\textsuperscript{17}, though normal limits for PWV in pregnancy have not been reported.

Furthermore, device manufacturers commonly state that it is necessary to standardise the time of the day when performing non-invasive cardiovascular assessments of arterial
stiffness. However, previous studies of diurnal variability of arterial stiffness have been limited to healthy non-pregnant populations\textsuperscript{18-20} with inconsistent methodologies. Only one small (n=15) study of diurnal variation in PWV used four time points and reported a lack of significant circadian rhythm changes\textsuperscript{20}. In addition, the repeatability of PWV has only been assessed in non-pregnant women\textsuperscript{19,21,22}. However, understanding the circadian pattern of maternal haemodynamics in normal pregnancy is crucial to both clinicians and researchers, as it may influence both the reliability and performance in screening for adverse pregnancy outcome. Therefore, the aim of the present study was to examine the repeatability of successive non-invasive cardiovascular measurements among pregnant women, and to assess diurnal haemodynamic changes in uncomplicated pregnancy.

**METHODS**

**Non-invasive Cardiovascular Assessment**

The arterial stiffness measurements were obtained using a commercially available, validated platform that use established methodology, tried and tested in clinical populations, the Arteriograph\textsuperscript{®}. CO measurements were obtained using the Non-Invasive Cardiac Output Monitor (NICOM, Cheetah Medical, Portland, Oregon). In addition to values for CO including HR and stroke volume (SV), the NICOM device calculates the SV index (SVI), CO, the total peripheral resistance (TPR) and BP.

Three professionals who received appropriate training in the use of the Arteriograph\textsuperscript{®} and the NICOM\textsuperscript{®} devices recorded all data. Both devices were automated and this therefore reduced the risk of inter-observer variability.
Both studies were approved by the Stanmore National Research Ethics committee. Written informed consent was obtained from all participating women before their enrolment.

**Diurnal Variation**

Twenty-one low-risk pregnant women were recruited. Participants were excluded if they had one or more of the following conditions: a BMI >25kg/m\(^2\) at booking, multiple pregnancy, foetal anomalies, essential hypertension, pregnancy-induced hypertension, pre-eclampsia, pregnancy complicated with foetal growth restriction or small for gestational age, thyroid disease requiring medication, renal disease, diabetes mellitus or on any medication that could affect BP. Participants were inpatients at the hospital for the duration of the study.

Participants were investigated at four time points during a 24-hour cycle: morning (between 0900 to 1000h), afternoon (1400-1500h), evening (2000-2100h), and midnight (0000-0100h). Participants were assessed in a temperature-controlled room (22°C) in a semi-recumbent position on their hospital bed, more than 45 minutes after food intake, and having avoided caffeine and alcohol for 24 hours. Participants rested for a minimum of ten minutes prior to the non-invasive haemodynamic examination and did not speak or move when the measurements were being undertaken.

**Repeatability study**

Sixty-seven women were recruited. The same exclusion criteria as those mentioned above were applied. Participants were assessed in a temperature-controlled room (22°C) in a semi-recumbent position on a hospital bed at the antenatal clinic. Participants rested for a minimum of ten minutes prior to non-invasive haemodynamic examination. Thereafter,
three repeated non-invasive cardiovascular measurements at five-minute intervals were recorded. Participants were asked not to speak or move when the measurements were being undertaken.

**Statistical analysis**

Data from the diurnal study on the different Arteriograph® and NICOM® variables, measured on 21 pregnant women during the third trimester of pregnancy at four different time points (morning, afternoon, evening and midnight), were analysed using a linear mixed model incorporating time as a fixed effect and individual patient as a random effect.

Data from the repeatability study (n=67) were analysed using a separate linear mixed model for each of the Arteriograph® and NICOM® variables. The model incorporated time as fixed effects and individual patient as a random effect.

To assess the correlation between observations on the same patient for both studies, we estimated the intra-class correlation (ICC) coefficient as the ratio of between patient variability to the overall variability and obtained 95% confidence interval of ICC by sampling the data using the bootstrap-based approach. A predictor was considered statistically significant if the two-sided type I error rate was less than 5% (i.e. p<0.05). All statistical analyses were carried out using the R software version 3.3 with appropriate R packages (R Core Team, 2016).
RESULTS

DIURNAL VARIATION

Twenty-one low risk pregnant women of mean age 28.95 (SD=6.38) years with a mean gestational age of 34 (3.74) weeks fulfilled our inclusion and exclusion criteria and agreed to participate in the study. Demographic details of the study population are summarised in Table 1. The mean values of arterial stiffness, at four time points (morning, afternoon, evening and midnight), are presented in Table 2, and individual measurements at these time points with the corresponding box plots are presented in Figure 1. There were non-significant reductions in brachial and aortic augmentation index (Alx) values from the morning to evening with increases afterwards, though PWV values increased non-significantly in the afternoon before decreasing later (Table 2).

The mean values of non-invasive CO variables using NICOM® are presented in Table 2, with individual measurements in Figure 1. Among cardiac output variables measured, Mean Arterial Pressure (MAP), SV, SVI and TPR did not show any evidence of diurnal variation between four time points in pregnant women in their third trimester. However, we observed that the mean CO, Cardiac Index (CI) and HR varied from morning to midnight; the mean CO, HR and CI increased significantly in the afternoon compared with the corresponding mean morning measurements in a similar fashion to HR.

ICC estimates between measurements on the same subject showed excellent or good correlation for NICOM variables while all the Arteriograph® variables showed fair estimates of ICC (Table 3). A moderate to higher estimate of ICC suggests higher between-patient variability and lower within-patient variability.
REPEATABILITY STUDY

Sixty-seven women, of mean (SD) age 31.57 (6.09) years at a mean gestational age of 27.70 (2.29) weeks participated in the study to assess the repeatability of successive measurements of the Arteriograph® and NICOM® variables.

Outcomes from the linear mixed models showed no evidence (p>0.14) that mean values of Arteriograph (Alx and PWV) and NICOM (CO, CI, SV, SVI) differed at three successive measurements taken five minutes apart as evidenced by Table 3. Most variables showed approximately 0.2 to 1.9% changes in consecutive measurements.

ICC estimates between measurements on the same subject showed excellent correlation for NICOM variables, with the exception of stroke volume variation, where correlation was good. For the Arteriograph® variables, aortic PWV exhibited excellent ICC, and aortic Alx good (Table 3).

Triplicate measurements on each subject of Arteriograph® and NICOM® variables are presented graphically in Figure 2. Triplicate measurements on the same patient are joined by dotted lines to demonstrate the consistency in repeatable measurements for each patient. We also presented individual values along with box plots at each measurement points (Figure 3) illustrating similar central tendencies across three successive measurements (1, 2, 3) for each subject.
DISCUSSION

To the best of our knowledge, this is the first study to investigate diurnal variation and the repeatability of measurements of arterial stiffness and CO among women during their third trimester of pregnancy. There was no evidence of significant diurnal variability for the majority of arterial stiffness and CO parameters studied, with the exception of CO and CI. Furthermore, measurements of vascular stiffness and CO were repeatable, with good to excellent ICC estimates.

Findings of non-significant diurnal variation of PWV and Alx reaffirm the previous work carried out in non-pregnant population\textsuperscript{18-20}. Drager and colleagues reported no evidence of circadian rhythm in PWV at four time points (08h00, 12h00, 16h00 and 20h00), though in a small population (n=15)\textsuperscript{20}. Other groups studied diurnal variability at fewer time-points, Ter Avest et al\textsuperscript{18} on two occasions (09h00 and 14h00) and Yanlei et al\textsuperscript{19} (n=70) on three (09h00, 13h00 and 17h00), and reported no diurnal variation in PWV. For the non-invasive CO using the NICOM®, the statistically significant higher mean CO in the afternoon conforms to the normal expected physiological variation. The lack of diurnal variability in arterial stiffness parameters is important to determine, as the lack of standardisation of measurements by time of day has been previously criticised\textsuperscript{24-26}. In addition, we can be confident of a lack of diurnal variability as measurements were performed at four time points in a day, compared to other studies which used longer intervals\textsuperscript{18,19}. Also, the present study population for the diurnal variation study (n=21) was adequately powered to assess for any biologically important changes amongst this population group. Nonetheless, it is vital that all other
determinants such as maternal age, HR and MAP be carefully evaluated and adjusted for accordingly.

The repeatability study findings are also consistent with previous work carried out in non-pregnant women. Yanlei and colleagues examined changes in 7 subjects and reported that the coefficient of variation (CV) of the two repeated measurements of PWV, 5 minutes apart, was 6.1% with the absolute difference for the repeated measures being -0.151 m/s (95% CI: -2.022 -1.720) . Baulman et al. validated measurements of arterial stiffness between an oscillometric (Arteriograph®), tonometric (SphygmoCor) and piezo-electronic methods (Complior®). The authors observed that the variance within one session was the lowest (0.18 m/s) for the Arteriograph® compared with the Complior® (0.312 m/s) and the SphygmoCor® (0.363 m/s). Importantly, we only looked at the repeatability of measurements within a single visit, whereas Baulman et al. evaluated the trend further by looking at the reproducibility of measurements between two sessions, undertaken one week apart. They found the measurement errors for the repeat measurements’ were also the lowest for the Arteriograph® (1.18 m/s), as compared to the Complior® (1.55 m/s) and the SphygmoCor® (1.67 m/s). In the present study, we only assessed repeatability of measurements within a single visit, so as to reduce the possible bias of advancing gestation on maternal haemodynamics.

One limitation of the present study was that assessments were confined to the third trimester of pregnancy. Over the first two trimesters of pregnancy, CO gradually increases with the greatest increase occurring by 16 weeks of gestation. The rise in CO typically plateaus after 20 weeks of gestation but remains at that elevated level until the term.
Therefore, we intentionally chose to evaluate the circadian rhythm of CO and arterial stiffness in women in their third trimester. In addition, our inclusion criteria aimed to reduce the influence of a maternal age range on the haemodynamic parameters as Khalil et al. have demonstrated that PWV and systolic BP have a directly proportional relationship to maternal age. It is also important to stress that diurnal variations reported in the present study pertained to young healthy low-risk pregnant women in their third trimester. Therefore, these conclusions may not be valid for women in the first two trimesters or patients with pregnancy complications or co-morbidity. Slightly lower estimates of ICC for Arteriograph measurements also suggest that a study with bigger sample size might be warranted to consider higher variabilities for these measurements.

**CONCLUSION**

With the exception of CO, diurnal measurements of these variables in young healthy low-risk pregnant women in their third trimester did not change significantly from morning to midnight. Multiple consecutive measurements of vascular stiffness and non-invasive CO measurements on the same woman in the third trimester of pregnancy were highly correlated, confirming excellent repeatability of measurements. Given the increase in the use of non-invasive haemodynamic monitors, results have great implications for clinical research and application in clinical practice. We propose that it is not mandatory to measure PWV and Alx at the same time of day. However, standardising the time of day for non-invasive cardiovascular assessment may be beneficial in longitudinal studies. Further work is required to evaluate the diurnal variation in high-risk pregnancies such as those complicated by diabetes, hypertension and other cardiac risk factors.
REFERENCES


Table 1: Estimates of mean (standard deviation) of maternal characteristics of participants in the diurnal variation and repeatability study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value (n=21)</th>
<th>Value (n=67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>28.95 (6.38)</td>
<td>31.57 (6.09)</td>
</tr>
<tr>
<td>Maternal body surface area (m²)</td>
<td>1.62 (0.18)</td>
<td>1.86 (0.19)</td>
</tr>
<tr>
<td>Maternal height (cm)</td>
<td>158.4 (7.75)</td>
<td>161.42 (6.42)</td>
</tr>
<tr>
<td>Maternal weight (kg)</td>
<td>57.41 (10.41)</td>
<td>75.61 (18.68)</td>
</tr>
<tr>
<td>Maternal body mass index (kg/m²)</td>
<td>22.62 (2.91)</td>
<td>28.68 (6.49)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>34.14 (3.74)</td>
<td>27.70 (2.29)</td>
</tr>
</tbody>
</table>
Table 2: Estimates of mean, standard deviation and intraclass correlation coefficients of arterial stiffness and cardiac output measurements at four time points (morning, afternoon, evening, midnight) for 21 low risk patients.

<table>
<thead>
<tr>
<th>Time interval</th>
<th>Cardiac Output mL/min</th>
<th>Cardiac Index L/min/m²</th>
<th>Stroke volume</th>
<th>Stroke Volume Index ml/m²</th>
<th>Mean arterial blood pressure</th>
<th>Heart rate</th>
<th>Total peripheral resistance</th>
<th>Brachial Aix %</th>
<th>Aortic Aix %</th>
<th>Aortic PWV m/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morning (0900 to 1000h)</td>
<td>5.90 (1.33)</td>
<td>3.65 (0.58)</td>
<td>64.17 (16.10)</td>
<td>39.05 (7.29)</td>
<td>81.70 (5.47)</td>
<td>95 (12.20)</td>
<td>1167.62 (300.72)</td>
<td>-46.96 (41.49)</td>
<td>10.22 (15.62)</td>
<td>7.81 (2.05)</td>
</tr>
<tr>
<td>Afternoon (1400 to 1500h)</td>
<td>6.38 (1.49)</td>
<td>3.93 (0.68)</td>
<td>66.52 (17.85)</td>
<td>40.25 (7.65)</td>
<td>84.65 (10.64)</td>
<td>98 (13.10)</td>
<td>1110.20 (268.75)</td>
<td>-65.57 (19.88)</td>
<td>4.44 (10.07)</td>
<td>8.45 (1.68)</td>
</tr>
<tr>
<td>Evening (2000 to 2100h)</td>
<td>6.18 (1.43)</td>
<td>3.81 (0.65)</td>
<td>63.64 (15.85)</td>
<td>41.36 (7.1)</td>
<td>84.49 (7.8)</td>
<td>95 (12.50)</td>
<td>1161.93 (270.12)</td>
<td>-61.54 (21.59)</td>
<td>6.49 (10.92)</td>
<td>7.87 (1.74)</td>
</tr>
<tr>
<td>Midnight (0000 to 0100h)</td>
<td>5.80 (1.19)</td>
<td>3.57 (0.48)</td>
<td>66.16 (15.54)</td>
<td>40.67 (6.57)</td>
<td>81.43 (10.06)</td>
<td>88 (12.98)</td>
<td>1170.85 (314.03)</td>
<td>-57.75 (16.12)</td>
<td>8.40 (8.16)</td>
<td>7.64 (1.15)</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.5063</td>
<td>0.582</td>
<td>0.071</td>
<td>&lt;0.001</td>
<td>0.307</td>
<td>0.077</td>
<td>0.295</td>
<td>0.267</td>
</tr>
<tr>
<td>ICC (95% lower, upper CI)</td>
<td>0.92 (0.87, 0.95)</td>
<td>0.84 (0.72, 0.91)</td>
<td>0.90 (0.82, 0.94)</td>
<td>0.71 (0.52, 0.83)</td>
<td>0.76 (0.60, 0.84)</td>
<td>0.76 (0.60, 0.84)</td>
<td>0.87 (0.78, 0.92)</td>
<td>0.33 (0.20, 0.51)</td>
<td>0.34 (0.18, 0.54)</td>
<td>0.46 (0.25, 0.67)</td>
</tr>
</tbody>
</table>
Table 3: Estimates of mean, standard deviation and intraclass correlation coefficients of arterial stiffness and cardiac output measurements at three replicates for 67 subjects.

<table>
<thead>
<tr>
<th>Indices (Unit)</th>
<th>Cardiac Output mL/min</th>
<th>Cardiac Index L/min/m²</th>
<th>Stroke volume ml</th>
<th>Stroke Volume Index ml/m²</th>
<th>Brachial Alx %</th>
<th>Aortic Alx %</th>
<th>Aortic PWV m/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=67)</td>
<td>7.37 (1.49)</td>
<td>3.97 (0.57)</td>
<td>82.95 (18.67)</td>
<td>44.27 (8.44)</td>
<td>-64.6 (20.85)</td>
<td>4.32 (8.40)</td>
<td>8.11 (1.56)</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time 1 (0 min)</td>
<td>7.43 (1.50)</td>
<td>3.99 (0.59)</td>
<td>83.25 (18.98)</td>
<td>44.4 (7.99)</td>
<td>-65.78 (17.69)</td>
<td>4.09 (8.87)</td>
<td>8.14 (1.53)</td>
</tr>
<tr>
<td>Time 2 (5 min)</td>
<td>7.28 (1.61)</td>
<td>3.97 (0.58)</td>
<td>82.59 (19.03)</td>
<td>44.31 (8.36)</td>
<td>-66.84 (16.39)</td>
<td>3.80 (8.30)</td>
<td>8.09 (1.28)</td>
</tr>
<tr>
<td>Time 3 (10 min)</td>
<td>7.39 (1.35)</td>
<td>3.97 (0.56)</td>
<td>83 (18.27)</td>
<td>44.1 (9.07)</td>
<td>-60.85 (27.31)</td>
<td>5.15 (8.03)</td>
<td>8.11 (1.86)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.756</td>
<td>0.708</td>
<td>0.818</td>
<td>0.686</td>
<td>0.136</td>
<td>0.450</td>
<td>0.638</td>
</tr>
<tr>
<td>ICC (95% lower, upper CI)</td>
<td>0.80 (0.62, 0.90)</td>
<td>0.78 (0.64, 0.85)</td>
<td>0.91 (0.85, 0.94)</td>
<td>0.80 (0.63, 0.89)</td>
<td>0.49 (0.23, 0.76)</td>
<td>0.67 (0.40, 0.83)</td>
<td>0.79 (0.58, 0.91)</td>
</tr>
</tbody>
</table>
Figure 1: Measurements of Arteriograph and NICOM variables of 21 patients (points) at four time points (morning, afternoon, evening and midnight) with the corresponding box plots showing the median and interquartile range.

Points with the same colour represent data from the same patient.
Figure 2: Measurements of Arteriograph® and NICOM® variables of all 67 patients (points) over three successive measurements (1, 2, 3) where triplicate measurements on the same patient are joined by dotted lines to demonstrate the consistency in repeatable measurements. The bold red line connects the mean values at each of the measurement points.

Points and lines with the same colour represent data from the same patient.
Figure 3: Measurements of Arteriograph® and NICOM ® variables of all 67 patients (points) over three successive measurements (1, 2, 3) along with the corresponding box plots showing the median and interquartile range.

Br Aix: Brachial augmentation index; Ao Aix: Aortic augmentation index; Ao PWV: Aortic pulse wave velocity; CO: Cardiac output; CI: Cardiac Index; SVI: Stroke volume index.

Points with the same colour represent data from the same patient.