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# Middle cerebral artery blood flow velocities and pulsatility index and the cerebroplacental pulsatility ratio: longitudinal reference ranges and terms for serial measurements

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**KEYWORDS:** blood flow; cerebroplacental ratio; Doppler; fetus; middle cerebral artery; reference ranges

## ABSTRACT

***Objectives*** *To establish reference ranges suitable for serial assessments of the fetal middle cerebral (MCA) and umbilical (UA) artery blood flow velocities, pulsatility index (PI) and cerebroplacental pulsatility ratio and to provide terms for calculating conditional reference intervals suitable for individual serial measurements.*

***Methods*** *This was a longitudinal study of 161 singleton pregnancies. Using Doppler ultrasound, MCA and UA blood velocities and PI were determined three to five times at 3–5-week intervals over a gestational age range of 19–41 weeks. Polynomial regression lines for the 95$^{th}$, 50$^{th}$ and 5$^{th}$ percentiles were calculated for the peak systolic velocity (PSV), time-averaged maximum velocity (TAMXV), PI and cerebroplacental ratio. Terms for calculating conditional reference intervals were established.*

***Results*** *Based on 566 observations our new longitudinal reference ranges for fetal middle cerebral PSV, TAMXV and PI provided terms for calculating conditional reference intervals (i.e. predicting expected 95% confidence limits based on a previous measurement), and correspondingly for the cerebroplacental ratio ($n = 550$). The reference ranges were at some variance with those of previous cross-sectional studies. The narrow 95% confidence limits for the 5$^{th}$ and 95$^{th}$ percentiles ensured reliable ranges.*

***Conclusions*** *We have established longitudinal reference ranges appropriate for the serial assessment of MCA blood velocities and PI and cerebroplacental ratio. Particularly the terms for calculating conditional ranges based on a previous observation make this system more appropriate for longitudinal monitoring than are cross-sectional data.*



## INTRODUCTION

Evaluation of the cerebral blood flow in the fetus has become an integrated part of the assessment of high-risk pregnancies. The middle cerebral artery (MCA) has been studied extensively, and its Doppler recordings are incorporated regularly into the management of fetuses at risk of developing placental compromise and fetal anemia. In cases of intrauterine growth restriction (IUGR), clinical management is based primarily on the waveform analysis, i.e. the pulsatility index (PI), a low PI reflecting redistribution of cardiac output to the brain[1,2]. MCA peak systolic velocity (PSV) is used mainly for the prediction and management of fetal anemia[3–5]. However, high MCA-PSV has been shown to predict perinatal mortality better than does low MCA-PI in a group of IUGR fetuses[6]. The surveillance of fetuses at risk usually requires serial Doppler measurements, including that of the MCA.

The use of such parameters depends on appropriate reference ranges[7]. However, while several cross-sectional reference ranges are now in use[8–10], these are less suitable for serial observations because the appropriate reference ranges for serial measurements require longitudinal data[11]. A few longitudinal studies have been published, but they suffer from too few participants, or lack ranges for commonly used parameters, and none has developed conditional terms for repeat measurements[12–14].

Combining the Doppler waveform analysis of the MCA with that of the umbilical artery (UA) by a common cerebroplacental ratio, i.e. the ratio of their

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pulsatility indices has been suggested as a useful clinical simplification[15]. A low cerebroplacental ratio reflects redistribution of the cardiac output to the cerebral circulation and has been shown to improve accuracy in predicting adverse outcome compared with MCA or UA Doppler alone[16–20]. The ratio has increasingly been incorporated in the surveillance of the fetus at risk by repeating the assessment at intervals[21–23]. Reference ranges currently in use for the cerebroplacental ratio assessment[21,24–26] are based on cross-sectional studies and thus suitable for single observations[27,28]. For serial assessments, however, the reference ranges should be based on studies with a longitudinal design[11].

The purpose of this study was therefore to establish new longitudinal reference ranges for MCA and UA Doppler velocities and PI and the cerebroplacental ratio, and to provide terms for calculating conditional reference intervals suitable for individual serial measurements.

## METHODS

The study population of 161 women was recruited from the low-risk population to a prospective longitudinal observational study of the cerebral, splanchnic and umbilical arterial circulation. Here we present the results of the MCA, UA and the cerebroplacental ratio part of that project. The regional committee for medical research ethics approved the study protocol (REK Vest no. 203.03), and all participants gave prior informed written consent. Gestational age was assessed by ultrasound head biometry at 17–20 weeks of gestation[29]. Multiple pregnancy, fetal abnormalities, obstetric history of previous complications such as pregnancy-induced hypertension, IUGR and placental abruption excluded participation, as did the presence of diabetes or any general chronic disease. None of the fetuses was at risk of developing anemia. Each woman was examined three to five times, at 3–5-week intervals over a gestational age range of 19–41 weeks. Complications in pregnancy and birth, birth weight, placental weight, Apgar score, age, gender and mode of delivery were noted. A pediatrician examined the neonates once during the first 3 postnatal days, and any abnormality was noted.

Doppler ultrasound measurements were recorded using a 2–5, 4–8 or 2–7-MHz transabdominal transducer (Voluson 730 Expert, GE Medical systems, Kretz Ultrasound, Zipf, Austria). The high-pass filter was set to 70 Hz and in the majority of the sessions the mechanical and thermal indices were below 1.1 and 0.9, respectively, and they were always kept below 1.9 and 1.5. The MCA was visualized using color flow mapping in an axial section of the brain. The Doppler beam was directed along the MCA, and the sample volume (median, 3 mm; mean, 3.5 mm; range, 1–7 mm) was placed over the proximal section where the MCA emerges from the circle of Willis. When the MCA in the near field could not be interrogated, we used the MCA of the opposite side. The recordings were acquired in the absence of fetal breathing or body movements over at least three uniform heart cycles. The Doppler waveforms were traced automatically, the PSV and time-averaged maximum velocity (TAMXV) were determined, and the PI was calculated according to Gosling and King[30].

The waveforms from the UA were obtained in a free-floating loop of the umbilical cord using a corresponding technique with the insonation in alignment with the direction of the vessel. The velocities and PI were entered into the statistics and the cerebroplacental ratio was calculated as the ratio of the MCA- and UA-PI.

The number of participants needed to construct percentiles based on cross-sectional collected data (Nc) has been calculated to be about 15 per gestational week[31]. The corresponding number for a longitudinally designed study (Nl) is Nc/D, were D is the 'design factor', which has been calculated as 2.3 in studies on fetal size[7]. Thus, observations over 20 weeks correspond to $Nc = 300$ and $Nl = 130$. An anticipated overall success rate of 80% for Doppler measurements increased the study group to 160 participants. Multilevel modeling was used in order to calculate mean and percentiles for the MCA Doppler velocities and PI according to gestational age. To achieve normal distribution of outcome variables, we used Box–Cox power transformation. Fractional polynomial regression models[11] were fitted to the data in order to construct mean curves for PSV, TAMXV and PI according to gestational age. We used multilevel modeling, where the first level was the variance between measurements within the same fetus and the second was the variance between the participating women. The choice of fixed and random components in the models was based on gain in likelihood. The confidence interval of the mean was calculated from the standard error (SE) of the mean. To obtain approximate SEs around the 5$^{th}$ and 95$^{th}$ percentiles, transformed observations $\pm 1.645$ SD were regressed against gestational age. The regression lines equaled the 5$^{th}$ and 95$^{th}$ percentiles. The SE of the regression lines was used to obtain 95% confidence intervals around the 5$^{th}$ and 95$^{th}$ percentiles. The longitudinal design provided the necessary information for establishing terms for conditional reference values; i.e. for calculating the expected value and reference ranges conditional on a previous measurement[11]. Briefly, the conditional reference interval is calculated from the conditional mean and variance and the level-2 covariance; the level-1 covariance is assumed to be zero[11]. Formulae for conditional means and SDs are presented in Appendix S1 online. The intraobserver and interobserver variations were calculated with a paired sample *t*-test on the basis of 20 and 14 observations, respectively. The statistical analysis was carried out using SPSS (Statistical Package for the Social Sciences, SPSS Inc, Chicago, IL, USA) and the MlWin program (MlWin, Centre for Multilevel Modelling, University of Bristol, Bristol, UK).

## RESULTS

Measurements were obtained from all 161 participants: one woman had one set of measurements, two had two

**Table 1** Characteristics of the study population ($n = 161$)

| *Characteristic* | *Median (range) or %* |
|---|---|
| Maternal age at inclusion (years) | 29 (20–40) |
| Parity | 1 (0–5) |
| Para 0 (%) | 48.4 |
| BMI at first visit (kg/m$^2$) | 22.9 (18.1–40.8) |
| Smokers (%) | 2.5 |
| Gestational age at delivery (weeks) | 40.4 (35.4–42.6) |
| Mode of delivery spontaneous (%) | 77 |
| Induction of labor (%) | 5.6 |
| Vacuum extraction (%) | 3.7 |
| Forceps (%) | 3.1 |
| Cesarean section (%) | 10.6 |
| Placental weight (g) | 720 (350–1200) |
| Birth weight (g) | 3700 (2260–4980) |
| Apgar score at 5 min < 7 (%) | 0.62 |

BMI, body mass index.

sets, 18 had three, 126 had four and 14 had five. The median fetal age at first examination in the study was 23.3 (range, 19.3–28.4) weeks. Characteristics of the study population are presented in Table 1. All except three women were Caucasian. Two pregnancies were the result of *in-vitro* fertilization. Five women developed pre-eclampsia (3.1%) and three women delivered preterm (gestational age, 35–36 weeks). One fetus developed a supraventricular arrhythmia shortly before term, and another proved to have a single umbilical artery. There were 80 female and 81 male neonates. Participants who developed complications after enrolment in the study were not excluded.

We obtained measurements of the MCA in 604 of 633 sessions, giving a success rate of 95.4%. Measurements that required an angle correction larger than 10° were not included in the statistics. No angle correction was required in 540 recordings and in 26 the angle correction was $\leq 10°$, leaving 566 observations for statistical analysis. Unfavorable fetal position or movements were the reasons for not achieving an acceptable measurement.

The results showed notable individual variation with gestational age (Figure S1, available online). The results of the MCA-PSV and PI with fitted mean and reference intervals are presented in Figures 1 and 2. The corresponding gestational age-specific reference values for the 2.5$^{th}$, 5$^{th}$, 10$^{th}$, 25$^{th}$, 50$^{th}$, 75$^{th}$, 90$^{th}$, 95$^{th}$ and 97.5$^{th}$ percentiles are presented in Tables 2 and 3 (Table S1 and Figure S2 for the TAMXV are available online). MCA velocities increased throughout the second half of pregnancy. The PSV increased less during the last 10 weeks compared with the TAMXV, resulting in a continuous decline in PI in the last 10 weeks of gestation. This gave the PI reference curve an inverted U-shape with a turning point at 30 weeks (Figure 2).

Terms for calculating conditional mean, SD and ranges are presented in Appendix S1 online. A clinical case illustrates the use of conditional ranges in serial measurements in Figure 3. A spreadsheet for calculating conditional mean and ranges (see Figure 3b) can be downloaded from the UOG website (Spreadsheet S1).

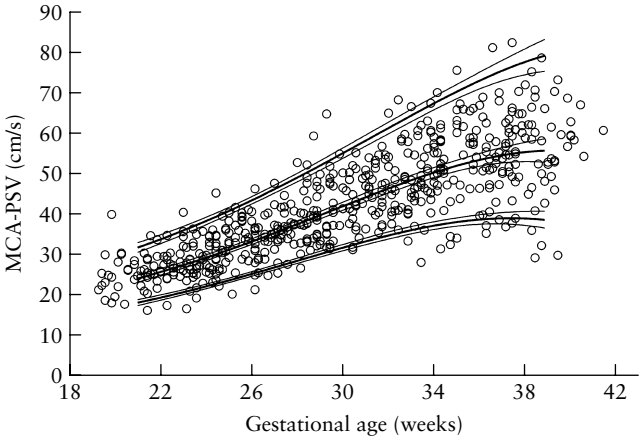



**Figure 1** Peak systolic velocity (PSV) in the middle cerebral artery (MCA) in 161 low-risk pregnancies (566 observations) with 5$^{th}$, 50$^{th}$ and 95$^{th}$ percentiles (thick lines) and their corresponding 95% CI (thin lines).

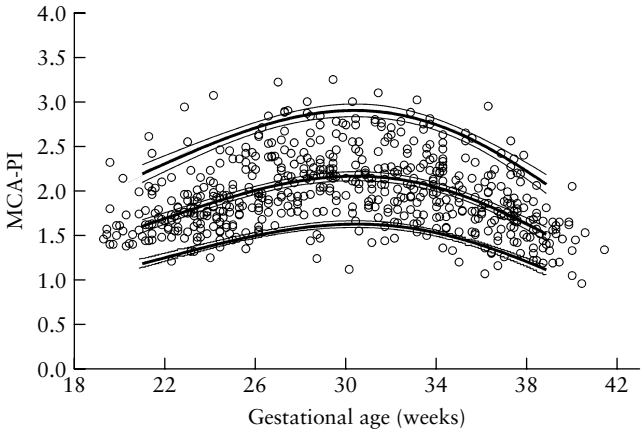



**Figure 2** Pulsatility index (PI) in the middle cerebral artery (MCA) in 161 low-risk pregnancies (566 observations) with 5$^{th}$, 50$^{th}$ and 95$^{th}$ percentiles (thick lines) and their corresponding 95% CI (thin lines).

The intra- and interobserver study showed acceptable reproducibility (Table 4).

We obtained 611 recordings of the UA velocity, and 550 complete sets of both MCA and UA observations. The angle of insonation of the UA was zero degrees in 582 observations and the median was 2 degrees in the remaining 29 (range 1–17). The UA-PI declined linearly from mean 1.15 at 21 weeks to 0.77 at 39 weeks (Figure 4). Individual observations of the umbilical artery peak systolic and time averaged maximum velocities and calculated 5$^{th}$, 50$^{th}$ and 95$^{th}$ percentiles with 95% CI are presented in Figures 5 and 6.

The cerebroplacental ratio increased from mean 1.41 at 21 weeks of gestation to a peak 2.36 at 33 weeks, and then decreased to 1.97 at 39 weeks (Figure 7). The 95% CI for the 5$^{th}$ percentile at 21 and 39 weeks were 0.84–0.96 and 1.12–1.40 respectively, and correspondingly for the 95$^{th}$ percentile 2.00–2.23 and 2.74–3.07. Gestational age specific values for the 2.5$^{th}$, 5$^{th}$, 10$^{th}$, 50$^{th}$, 75$^{th}$, 90$^{th}$, 95$^{th}$ and 97.5$^{th}$ percentiles for the cerebroplacental ratio are presented in Table 5. In Figure 8 a clinical example

**Table 2** Longitudinal reference ranges for the middle cerebral artery peak systolic velocity (in cm/s) based on 566 observations in 161 low-risk pregnancies

| GA (weeks) | Percentile | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2.5th | 5th | 10th | 25th | 50th | 75th | 90th | 95th | 97.5th |
| 21 | 17.14 | 18.12 | 19.31 | 21.46 | 24.09 | 27.00 | 29.90 | 31.75 | 33.45 |
| 22 | 18.34 | 19.37 | 20.63 | 22.91 | 25.69 | 28.77 | 31.83 | 33.79 | 35.57 |
| 23 | 19.62 | 20.72 | 22.05 | 24.47 | 27.41 | 30.67 | 33.90 | 35.97 | 37.86 |
| 24 | 20.98 | 22.15 | 23.56 | 26.12 | 29.25 | 32.70 | 36.12 | 38.31 | 40.31 |
| 25 | 22.41 | 23.65 | 25.16 | 27.87 | 31.19 | 34.85 | 38.48 | 40.80 | 42.91 |
| 26 | 23.89 | 25.21 | 26.82 | 29.70 | 33.22 | 37.11 | 40.96 | 43.42 | 45.67 |
| 27 | 25.43 | 26.83 | 28.53 | 31.60 | 35.34 | 39.47 | 43.56 | 46.18 | 48.56 |
| 28 | 26.98 | 28.47 | 30.28 | 33.54 | 37.52 | 41.92 | 46.27 | 49.05 | 51.59 |
| 29 | 28.53 | 30.11 | 32.04 | 35.51 | 39.74 | 44.42 | 49.06 | 52.03 | 54.73 |
| 30 | 30.04 | 31.73 | 33.77 | 37.47 | 41.98 | 46.97 | 51.91 | 55.08 | 57.97 |
| 31 | 31.49 | 33.28 | 35.46 | 39.39 | 44.19 | 49.51 | 54.79 | 58.18 | 61.27 |
| 32 | 32.83 | 34.73 | 37.04 | 41.22 | 46.34 | 52.02 | 57.67 | 61.30 | 64.61 |
| 33 | 34.02 | 36.04 | 38.49 | 42.94 | 48.39 | 54.46 | 60.50 | 64.39 | 67.94 |
| 34 | 35.02 | 37.16 | 39.76 | 44.48 | 50.29 | 56.77 | 63.24 | 67.41 | 71.22 |
| 35 | 35.79 | 38.05 | 40.80 | 45.81 | 51.99 | 58.90 | 65.83 | 70.31 | 74.41 |
| 36 | 36.29 | 38.66 | 41.57 | 46.86 | 53.43 | 60.81 | 68.22 | 73.02 | 77.43 |
| 37 | 36.48 | 38.97 | 42.02 | 47.60 | 54.56 | 62.41 | 70.34 | 75.49 | 80.23 |
| 38 | 36.33 | 38.92 | 42.12 | 47.99 | 55.34 | 63.67 | 72.13 | 77.64 | 82.73 |
| 39 | 35.82 | 38.51 | 41.83 | 47.97 | 55.70 | 64.52 | 73.52 | 79.41 | 84.85 |

GA, gestational age.

**Table 3** Longitudinal reference ranges for the middle cerebral artery pulsatility index based on 566 observations in 161 low-risk pregnancies

| GA (weeks) | Percentile | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2.5th | 5th | 10th | 25th | 50th | 75th | 90th | 95th | 97.5th |
| 21 | 1.12 | 1.18 | 1.26 | 1.41 | 1.60 | 1.82 | 2.04 | 2.19 | 2.33 |
| 22 | 1.18 | 1.25 | 1.33 | 1.49 | 1.69 | 1.92 | 2.15 | 2.30 | 2.45 |
| 23 | 1.24 | 1.32 | 1.41 | 1.57 | 1.78 | 2.01 | 2.25 | 2.41 | 2.56 |
| 24 | 1.31 | 1.38 | 1.47 | 1.64 | 1.86 | 2.10 | 2.35 | 2.52 | 2.67 |
| 25 | 1.36 | 1.44 | 1.54 | 1.71 | 1.94 | 2.19 | 2.45 | 2.62 | 2.78 |
| 26 | 1.42 | 1.50 | 1.60 | 1.78 | 2.01 | 2.26 | 2.53 | 2.71 | 2.87 |
| 27 | 1.46 | 1.55 | 1.65 | 1.83 | 2.06 | 2.33 | 2.60 | 2.78 | 2.95 |
| 28 | 1.50 | 1.58 | 1.69 | 1.88 | 2.11 | 2.38 | 2.66 | 2.84 | 3.01 |
| 29 | 1.53 | 1.61 | 1.71 | 1.91 | 2.15 | 2.42 | 2.70 | 2.88 | 3.05 |
| 30 | 1.54 | 1.62 | 1.73 | 1.92 | 2.16 | 2.44 | 2.72 | 2.90 | 3.07 |
| 31 | 1.54 | 1.62 | 1.73 | 1.92 | 2.16 | 2.43 | 2.71 | 2.90 | 3.07 |
| 32 | 1.52 | 1.61 | 1.71 | 1.90 | 2.14 | 2.41 | 2.69 | 2.87 | 3.04 |
| 33 | 1.49 | 1.58 | 1.68 | 1.87 | 2.10 | 2.37 | 2.64 | 2.82 | 2.98 |
| 34 | 1.45 | 1.53 | 1.63 | 1.81 | 2.04 | 2.30 | 2.57 | 2.74 | 2.90 |
| 35 | 1.39 | 1.47 | 1.56 | 1.74 | 1.96 | 2.21 | 2.47 | 2.64 | 2.80 |
| 36 | 1.32 | 1.39 | 1.48 | 1.65 | 1.86 | 2.11 | 2.36 | 2.52 | 2.67 |
| 37 | 1.23 | 1.30 | 1.39 | 1.55 | 1.75 | 1.98 | 2.22 | 2.38 | 2.52 |
| 38 | 1.14 | 1.20 | 1.29 | 1.44 | 1.63 | 1.85 | 2.07 | 2.22 | 2.36 |
| 39 | 1.04 | 1.10 | 1.18 | 1.32 | 1.49 | 1.70 | 1.91 | 2.05 | 2.18 |

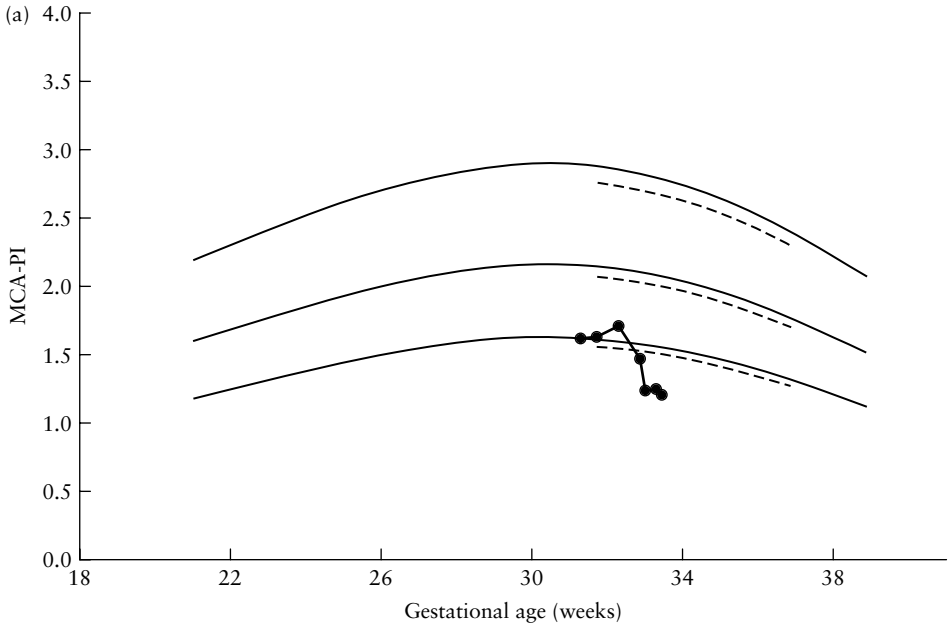
GA, gestational age.

of a high-risk pregnancy illustrates the calculation of conditional mean and ranges based on the first examination. All equations and terms are listed in the Appendix S2, and a spreadsheet for practical use is available online (Spreadsheet S2).

## DISCUSSION

We have established longitudinal reference ranges for the MCA-PSV, -TAMXV and -PI and the cerebroplacental ratio suitable for use with serial measurements for fetal surveillance. We have also provided terms for calculating conditional reference ranges needed for such longitudinal monitoring (Appendix S1 and S2 online). In practical terms, this means that the expected 50th percentile and corresponding ranges can be adjusted according to the previous observation, as shown in the examples in Figures 3 and 8. Typically, the conditional 50th percentile is shifted in the same direction as the index value, and




(b) PI

| Type: | | | Unconditional percentiles | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | weeks | days | 50 | 2.5 | 5 | 10 | 25 | 75 | 90 | 95 | 97.5 |
| GA 1st visit: | 31 | 2 | 2.16 | 1.54 | 1.62 | 1.73 | 1.92 | 2.43 | 2.71 | 0.92 | 3.06 |
| PI 1st visit: 1.62 | | | | | | | | | | | |
| GA today: | 31 | 5 | 2.15 | 1.53 | 1.61 | 1.72 | 1.91 | 2.42 | 2.70 | 2.88 | 3.05 |
| | | | Conditional percentiles | | | | | | | | |
| | | | 50 | 2.5 | 5 | 10 | 25 | 75 | 90 | 95 | 97.5 |
| | | | 2.07 | 1.48 | 1.56 | 1.66 | 1.84 | 2.33 | 2.59 | 2.76 | 2.92 |

**Figure 3** (a) Serial measurements of pulsatility index (PI) in the middle cerebral artery (MCA) in a fetus under surveillance for intrauterine growth restriction (●), conditional mean and reference interval (5th and 95th percentiles) calculated from the first measurement (– – –) and unconditional 5th, 50th and 95th percentiles (——). (b) Spreadsheet for the calculation of unconditional and conditional mean and percentiles. In this example, the conditional mean and ranges for week 31 + 5 were calculated based on the measurement at 31 + 2 weeks. 1st visit, values from previous measurements; GA, gestational age.

**Table 4** Intra- and interobserver variation in middle cerebral artery flow velocities and pulsatility index

| | *Paired differences* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *95% CI* | | | | | *LOA* | |
| | *Mean* | *SD* | *SEM* | *Lower* | *Upper* | t | *d.f.* | P* | *Lower* | *Upper* |
| Intraobserver variation | | | | | | | | | | |
| PSV | −0.24 | 1.14 | 0.26 | −0.78 | −0.31 | −0.90 | 18 | 0.380 | −2.47 | 2.00 |
| TAMXV | −0.16 | 0.79 | 0.18 | −0.54 | 0.22 | −0.88 | 18 | 0.390 | −1.71 | 1.39 |
| PI | 0.00 | 0.12 | 0.03 | −0.06 | 0.06 | 0.04 | 18 | 0.970 | −0.24 | 0.24 |
| Interobserver variation | | | | | | | | | | |
| PSV | 1.46 | 7.44 | 1.99 | −2.84 | 5.75 | 0.73 | 13 | 0.477 | −13.13 | 16.04 |
| TAMXV | 0.29 | 2.98 | 0.80 | −1.43 | 2.01 | 0.36 | 13 | 0.724 | −5.55 | 6.13 |
| PI | 0.05 | 0.43 | 0.11 | −0.20 | 0.30 | 0.43 | 13 | 0.674 | −0.79 | 0.89 |

*Two-tailed significance. d.f., degrees of freedom; LOA, limits of agreement; PI, pulsatility index; PSV, peak systolic velocity; SEM, standard error of the mean; *t*, test value of the paired *t*-test; TAMXV, time-averaged maximum velocity.

the ranges are narrower compared with the unconditional ones. This provides an objective method of assessing changes in blood flow, which cross-sectional percentiles cannot achieve with validity.

The reference ranges we have established for the MCA differ slightly from those of cross-sectional studies. Our PSV values are slightly lower compared with those of Kurmanavicius *et al.*[9], especially after 34 weeks of gestation (Figure 9). Compared with Mari *et al.*[10], our mean values are higher from the 28th to the 35th week of gestation. Our finding that the increase in velocity is blunted after 36 weeks of gestation is in line with

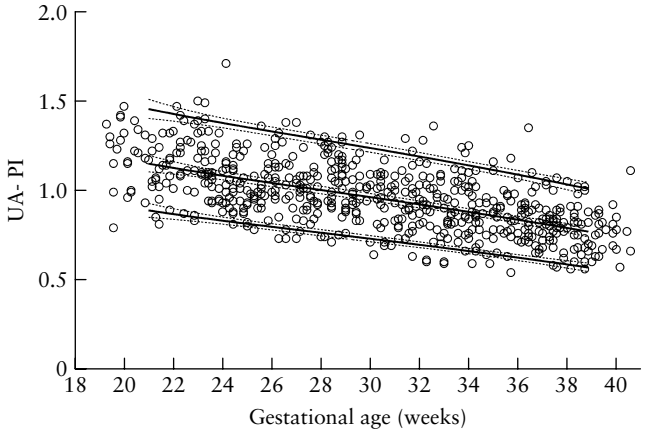



**Figure 4** Umbilical artery pulsatility index (UA-PI) (free loop) in 161 low-risk pregnancies (611 observations) with 5th, 50th and 95th percentiles (solid lines) and their corresponding 95% CI (dashed lines).

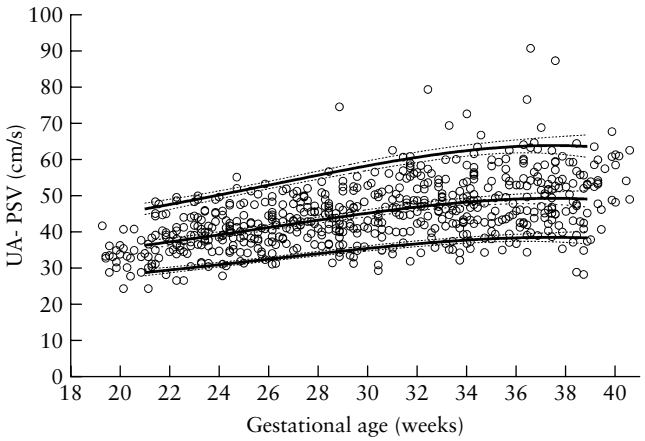



**Figure 5** Umbilical artery peak systolic velocity (UA-PSV) (free loop) in 161 low-risk pregnancies (611 observations) with 5th, 50th and 95th percentiles (solid lines) and their corresponding 95% CI (dashed lines).

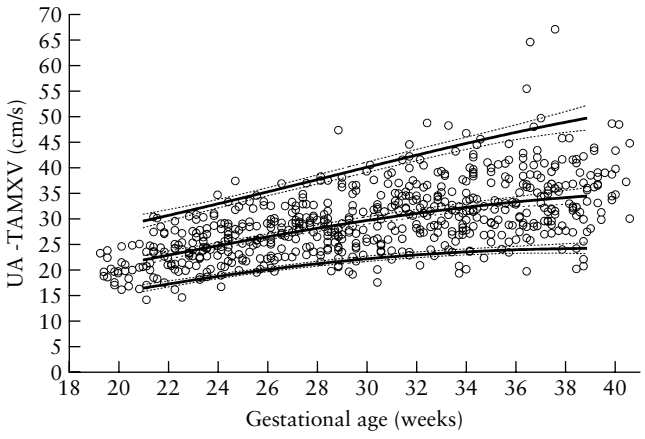



**Figure 6** Umbilical artery time averaged maximum velocity (UA-TAMXV) (free loop) in 161 low-risk pregnancies (611 observations) with 5th, 50th and 95th percentiles (solid lines) and their corresponding 95% CI (dashed lines).

another small longitudinal study[14], and probably reflects a physiological reduction of vascular impedance.

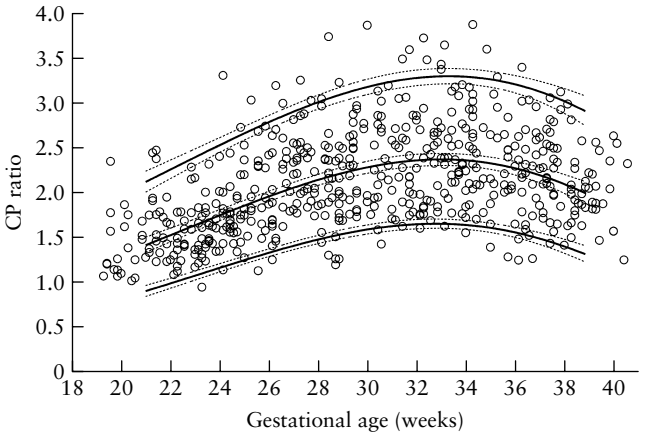



**Figure 7** Cerebroplacental ratio (CP ratio) in 161 low-risk pregnancies (550 observations) with 5th, 50th and 95th percentiles (solid lines) and their corresponding 95% CI (dashed lines).

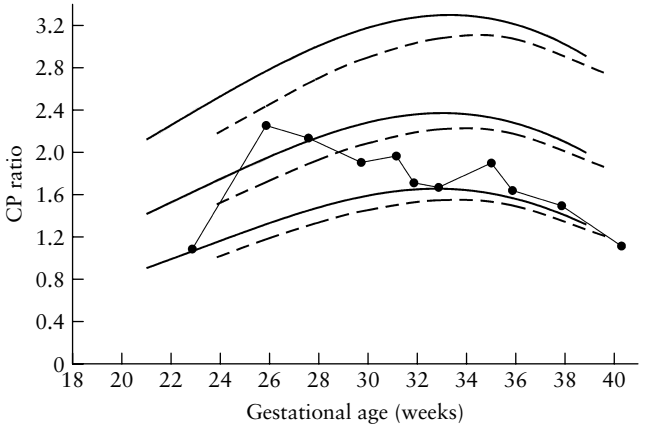



**Figure 8** Serial measurements of the cerebroplacental ratio (CP ratio) in a pregnancy at risk of intrauterine growth restriction (●). Based on Doppler measurements and cardiotocography she was delivered acutely at term of a girl weighing < 2.5th percentile (2250 g). Conditional percentiles (– – –) were calculated from the first observation at gestational age 22.9 weeks. Unconditional reference ranges are also shown (——). The necessary terms for calculating the conditional mean and ranges are found in Appendix S2, and a spreadsheet for practical use is available online.

The method of using multiples of the median for the MCA-PSV has been extremely successful in identifying fetal anemia using single observations[5]. To further improve surveillance, Detti *et al.*[32] suggested the steepness of a regression line constructed from three successive measurements would indicate the severity of the development. However, to take full advantage of serial measurements, the calculation of longitudinally conditioned ranges, such as in our present study, offers a more statistically sound basis for predicting a deviation from normal development.

Our parabolic curves for PI in the MCA are higher than are those of Bahlmann[8], and somewhat lower than are those of Mari and Deter[2] (Figure 10). However, all the compared reference curves had similar values for the last weeks of pregnancy. Study methodology was expected to cause differences between our results

**Table 5** Longitudinal reference ranges for the cerebroplacental Doppler ratio (formed as a ratio of the middle cerebral and umbilical artery pulsatility index) based on 550 observations in 161 low-risk pregnancies

| *GA (weeks)* | *Percentile* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *2.5th* | *5th* | *10th* | *25th* | *50th* | *75th* | *90th* | *95th* | *97.5th* |
| 21 | 0.82 | 0.90 | 1.00 | 1.18 | 1.41 | 1.67 | 1.94 | 2.11 | 2.27 |
| 22 | 0.90 | 0.98 | 1.09 | 1.28 | 1.52 | 1.79 | 2.07 | 2.25 | 2.42 |
| 23 | 0.98 | 1.07 | 1.18 | 1.38 | 1.63 | 1.92 | 2.20 | 2.39 | 2.56 |
| 24 | 1.06 | 1.16 | 1.27 | 1.48 | 1.74 | 2.04 | 2.33 | 2.52 | 2.70 |
| 25 | 1.14 | 1.24 | 1.36 | 1.58 | 1.85 | 2.15 | 2.46 | 2.65 | 2.83 |
| 26 | 1.22 | 1.32 | 1.45 | 1.67 | 1.95 | 2.26 | 2.58 | 2.78 | 2.96 |
| 27 | 1.30 | 1.40 | 1.53 | 1.76 | 2.05 | 2.37 | 2.69 | 2.90 | 3.08 |
| 28 | 1.37 | 1.47 | 1.60 | 1.84 | 2.14 | 2.46 | 2.79 | 3.00 | 3.19 |
| 29 | 1.42 | 1.53 | 1.67 | 1.91 | 2.21 | 2.55 | 2.88 | 3.09 | 3.29 |
| 30 | 1.47 | 1.58 | 1.72 | 1.97 | 2.28 | 2.62 | 2.95 | 3.17 | 3.37 |
| 31 | 1.51 | 1.62 | 1.76 | 2.01 | 2.32 | 2.67 | 3.01 | 3.23 | 3.43 |
| 32 | 1.53 | 1.64 | 1.78 | 2.04 | 2.35 | 2.70 | 3.05 | 3.27 | 3.47 |
| 33 | 1.53 | 1.65 | 1.79 | 2.05 | 2.36 | 2.72 | 3.07 | 3.29 | 3.49 |
| 34 | 1.52 | 1.63 | 1.78 | 2.04 | 2.35 | 2.71 | 3.06 | 3.29 | 3.49 |
| 35 | 1.49 | 1.60 | 1.74 | 2.00 | 2.32 | 2.68 | 3.03 | 3.26 | 3.46 |
| 36 | 1.44 | 1.55 | 1.69 | 1.95 | 2.27 | 2.62 | 2.97 | 3.20 | 3.41 |
| 37 | 1.37 | 1.48 | 1.62 | 1.88 | 2.19 | 2.54 | 2.89 | 3.12 | 3.33 |
| 38 | 1.29 | 1.40 | 1.53 | 1.78 | 2.09 | 2.44 | 2.79 | 3.01 | 3.22 |
| 39 | 1.19 | 1.29 | 1.43 | 1.67 | 1.97 | 2.31 | 2.66 | 2.88 | 3.09 |

GA, gestational age.

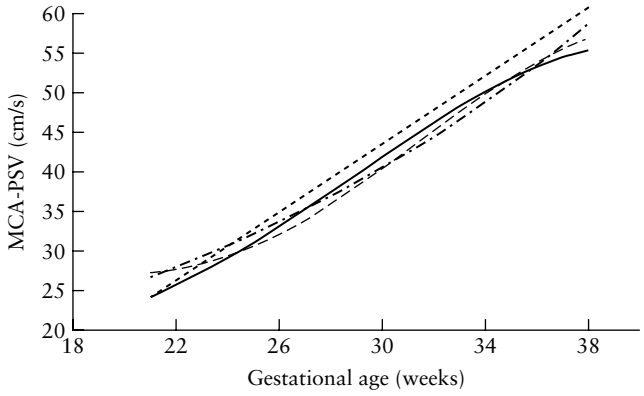



**Figure 9** Mean peak systolic velocity (PSV) in the middle cerebral artery (MCA) in our present longitudinal study (——) compared with that in the cross-sectional studies of Kurmanavicius *et al.*[9] (·····), Mari *et al.*[10] (·–·–) and Bahlmann[8] (– – –).

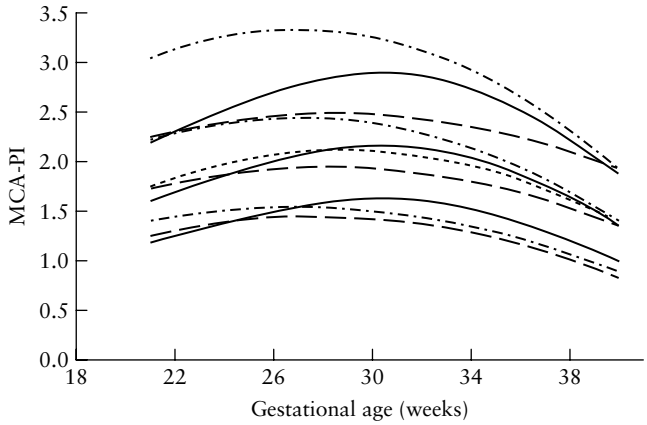



**Figure 10** Reference ranges (5th, 50th and 95th percentiles) for pulsatility index (PI) in the middle cerebral artery (MCA) in our present longitudinal study (——) compared with those in the cross-sectional studies of Bahlmann[8] (— –), Mari and Deter[2] (mean and predicted values, ·–·–) and Baschat[25] (mean, - - - -).

and those of others. The study on MCA-PSV by Mari *et al.*[10] was retrospective and included 135 participants at 15–42 gestational weeks, whilst the study by Mari and Deter[2] on PI included 128 participants, which, according to Royston and Altman[7], is too small a number from which to construct reliable reference ranges. The study by Kurmanavicius *et al.*[9] on MCA-PSV had few observations for the last weeks of pregnancy, whilst Bahlmann[8] had few observations in the early weeks and excluded fetuses with biometric parameters of the head and abdomen outside the 90% reference interval. The 95% CIs for the 5th, 50th and 95th percentiles presented here (Figures 1 and 2) assured reliable ranges for clinical use, both for single measurements and for longitudinal surveillance, provided that the same technique of measurement is applied.

The variability of velocities and indices has been shown to be larger and the PI higher in the distal portion compared with the proximal third of the MCA[33–36]. We therefore standardized our technique to record at the origin of the vessel at the circle of Willis, and we recommend an insonation aligned accurately along the vessel. In our hands, this technique gave reproducible results, with no difference between observers and acceptable limits of agreement (Table 4). We measured TAMXV because this parameter is robust and is influenced less by interference compared with weighted mean velocity[37]. A retrospective study by Bartha *et al.*[38] showed that time-averaged weighted mean velocity is altered earlier than is PSV when

anemia develops. We assume that TAMXV could be a corresponding useful parameter for surveillance of fetuses at risk of anemia.

Our longitudinal reference ranges for the cerebroplacental ratio are suitable both for single observations (Figure 7 and Table 5) and for serial measurements (when using the corresponding terms as shown in Figure 8). The number of included participants and observations ensured reliable ranges as reflected in the narrow 95% CI for the 5th, 50th and 95th percentiles (Figure 7). Our results differ somewhat from reference curves based on cross-sectional data (Figure 11)[21,24,25]. Low numbers of observations (82)[21], or unknown method of the MCA measurement[24], and different site of MCA recording (i.e. distal section of the vessel[25] rather than the recommended proximal site,[33,36,39]) may have contributed to the variation between curves. The difference in design and analysis was also expected to cause visible differences. When comparing our results of the UA velocities with those of another longitudinal study with corresponding design and insonation technique[40] we found minor differences, while the results of the UA-PI were identical, which was a reassuring external validation.

Different approaches have been applied in order to interpret cerebroplacental ratio results, a fixed cut-off at = 1.08[16] or cross-sectional reference ranges according to gestational age[21,24,25]. Curiously, one study showed that the use of a uniform cut-off for the second half of pregnancy (Figure 11) predicts adverse outcome equally well compared with using reference ranges according to gestational age[23]. Cerebroplacental ratio performs differently before and after 34 weeks of gestation[21,23], which may reflect differences in physiologic responses with advancing development. We believe that by introducing our new longitudinal reference ranges and individually calculated conditional ranges for repeat observations, the clinical application of the cerebroplacental ratio is more in line with physiologic development.

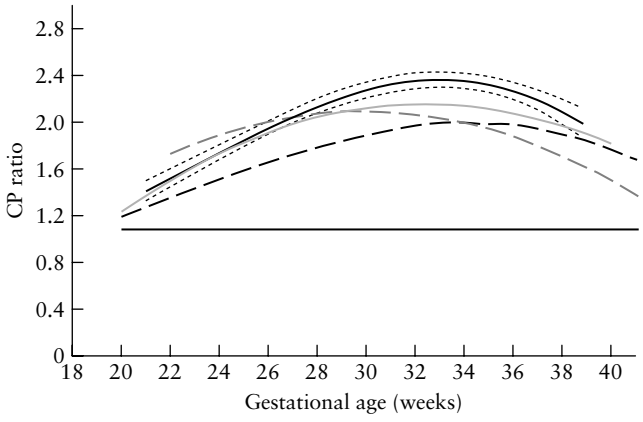



**Figure 11** The 50th percentile for the cerebroplacental ratio (CP ratio) established in the present longitudinal study (——) with 95% CI (·····) compared with cross-sectional studies: Baschat and Gembruch[25] (——), Arduini and Rizzo[24] (– – –), Bahado-Singh *et al.*[21] (— — —), and categorical cut-off 1.08 (——) according to Gramellini *et al.*[16].

The hypoxic index (amplitude of flow redistribution × duration in days) was introduced by Arbeille *et al.* in order to describe the cumulative intensity and duration of hypoxia or redistribution of flow to the brain in fetuses at risk[41]. The approach seems intricate and time-consuming. Our alternative, using a spreadsheet to calculate conditional ranges for repeat assessments of the cerebroplacental ratio appears quicker and is individually adjusted.

Our aim was to construct reference ranges for the second half of pregnancy. However, we obtained insufficient numbers of observations for constructing reliable ranges for gestational age below 21 and above 39 weeks (Figures 1, 2 and 7). A considerably larger study population would be needed to cover the period of 40–42 weeks of gestation adequately, a period when a substantial proportion of the women has delivered. We also aimed to examine each participant at least three times, but one woman had only one examination, and two had two examinations. Nevertheless, the study population showed overall excellent compliance.

The question may be raised as to whether our reference ranges were applicable to the background population. The study was based on a population that was at low risk. The gestational age at delivery (median, 40.4 weeks), average birth weight (mean 3660 g[42]) and Cesarean section rate (approximately 12%) were in accordance with that of the background population. By not excluding participants who developed complications, we avoided selection towards the supernormal and believe that our reference ranges can be applied in a general population.

It can be argued that the study population was rather uniformly of Nordic ethnicity and that the reference ranges may not be equally applicable in different ethnic populations. Apart from birth weight and placental size, it has been shown that the effect of ethnic and socioeconomic differences is likely to be small[43]. Since our data are longitudinally designed, conditional reference ranges can be calculated for any fetus based on a previous measurement.

In short, we have established new reference ranges for MCA-PSV, -TAMXV and -PI as well as for the cerebroplacental ratio based on longitudinal observations and have provided terms for calculating the conditional reference ranges that are needed for longitudinal monitoring.

## ACKNOWLEDGMENT

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## SUPPLEMENTARY MATERIAL ON THE INTERNET

The following material is available from the Journal homepage:

http://www.interscience.wiley.com/jpages/0960-7692/suppmat (restricted access)

**Figure S1** Raw data (566 observations) for the middle cerebral artery peak systolic blood velocity (PSV) in 161 low-risk pregnancies, illustrating the longitudinal variation in the population. Each line represents results from one fetus.

**Figure S2** Individual observations ($n = 566$) of time-averaged maximum velocity (TAMXV) in the middle cerebral artery in 161 low-risk pregnancies, with fitted $5^{th}$, $50^{th}$ and $95^{th}$ percentiles. Thin lines represent 95% confidence limits for these percentiles.

**Table S1** Longitudinal reference ranges for the middle cerebral artery time-averaged maximum velocity (cm/s) based on 566 observations in 161 low-risk pregnancies.

**Spreadsheet S1** Excel spreadsheet for calculating unconditional and conditional ranges for serial measurements of middle cerebral artery blood flow velocities and pulsatility index.

**Spreadsheet S2** Excel spreadsheet for calculating unconditional and conditional ranges for serial measurements of cerebroplacental pulsatility ratio.

**Appendix S1** Formulae for calculating conditional means, reference ranges and SDs for serial measurements of middle cerebral artery blood flow velocities and pulsatility index.

**Appendix S2** Formulae for calculating conditional means, reference ranges and SD for serial measurements of umbilical artery blood flow velocities and pulsatility index and the cerebroplacental ratio.

 