Brain sparing effect in growth-restricted fetuses is associated with decreased cardiac acceleration and deceleration capacities: a case–control study

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Objective Phase rectified signal averaging (PRSA) is a new method of fetal heart rate variability (fHRV) analysis that quantifies the average acceleration (AC) and deceleration capacity (DC) of the heart. The aim of this study was to evaluate AC and DC of fHR [recorded by trans-abdominal fetal electrocardiogram (ta-fECG)] in relation to Doppler velocimetry characteristics of intrauterine growth restriction (IUGR).

Design Prospective case–control study.

Setting Single third referral centre.

Population IUGR (n = 66) between 25 and 40 gestational weeks and uncomplicated pregnancies (n = 79).

Methods In IUGR the nearest ta-fECG monitoring to delivery was used for PRSA analysis and Doppler velocimetry parameters obtained within 48 hours. AC and DC were computed at $s = T = 9$. The relation was evaluated between either AC or DC and Doppler velocimetry parameters adjusting for gestational age at monitoring, as well as the association between either AC or DC and IUGR with or without brain sparing.

Results In IUGRs there was a significant association between either AC and DC and middle cerebral artery pulsatility index (PI; $P = 0.01$; $P = 0.005$), but the same was not true for uterine or umbilical artery PI ($P > 0.05$). Both IUGR fetuses with and without brain sparing had lower AC and DC than controls, but this association was stronger for IUGRs with brain sparing.

Conclusions Our study observed for the first time that AC and DC at PRSA analysis are associated with middle cerebral artery PI, but not with uterine or umbilical artery PI, and that there is a significant decrease of AC and DC in association with brain sparing in IUGR fetuses from 25 weeks of gestation to term.

Keywords Doppler velocimetry, fetal heart rate variability, intrauterine growth restriction, phase rectified signal averaging.

Tweetable abstract Brain sparing in IUGR fetuses is associated with decreased acceleration and deceleration capacities of the heart.

Introduction

Intrauterine growth restriction (IUGR) is a complex process of adaptation of the growing fetus to the restricted metabolic supply of the placenta, unable to negotiate the full requirements of fetal genetic potential. Each metabolic pathway, organ and function reshapes a strategy to cope with this deprived environment. These processes of fetal programming will possibly influence the short- and long-term health of the newborn, and of the adult to be.

In human pregnancy we have limited access to these processes by means of biophysical non-invasive diagnostic

tools. Yet, timing of delivery in IUGR relies on these proxies, and should reach the best possible balance between adaptation, gestational age and permanent damage.

Cardiovascular adaptation to placental insufficiency can be tracked by sequential Doppler changes, whose meaning varies according to gestational age.[1] Among these proxies, the pulsatility index (PI) of the middle cerebral artery identifies a process of adaptation by dilatation, the brain sparing effect.[2] Low middle cerebral artery PI correlates with fetal outcome not only in early severe cases flagged by an abnormal umbilical arterial PI, but also in late and term IUGR in which umbilical artery PI might be normal.[3]

The evaluation of central nervous system adaptation might be further improved by the assessment of the reactivity of the autonomous nervous system (ANS) as mirrored by fetal heart rate variability (fHRV). However, the standard methods of fHRV monitoring are unable to capture the true beat-to-beat variability that reflects the ANS control of the fHR due to intrinsic limitations (i.e. low sampling frequency and averaging process). Thus, alternative monitoring methods that might overcome these limitations are still needed.

Phase rectified signal averaging (PRSA) is a new method of analysis of complex biological signals. It has been developed in adult cardiology and has proved to be superior to standard methods of monitoring.[4] The fHR signal processed by PRSA is then employed to quantify the average acceleration (AC) and deceleration capacity (DC) of the heart. The technique has several advantages: it is more robust to non-stationarities, artefacts and noise than standard methods.[5]

In our previous studies we found that with acute hypoxic insult (i.e. pregnant sheep model exposed to repetitive cord occlusions), the AC and DC increase,[6] whereas during chronic hypoxia (i.e. human IUGR fetuses) the AC and DC decrease.[7] Thus, PRSA analysis is capable of capturing the ANS activation in the case of acute hypoxic insult, and to identify lower ANS reactivity in IUGRs confirming the potential value of the PRSA method for fetal monitoring.[8]

The aim of this study was to evaluate the AC and DC of fHR [recorded by trans-abdominal fetal electrocardiogram (ta-fECG)] in relation to Doppler velocimetry characteristics of IUGR fetuses, especially with regard to the middle cerebral artery PI.

Material and methods

Study design and population

This single-centre prospective case–control study was conducted at the Department of Obstetrics and Gynaecology, Children's Hospital Vittore Buzzi, University of Milan, Italy. We recruited pregnancies admitted to the high-risk ward with a diagnosis of fetal IUGR between 25 and 40 weeks of gestation. Multiple pregnancies and pregnancies with fetuses with chromosomal or structural anomalies were excluded. The controls were uncomplicated pregnancies with a distribution of gestational age equivalent to that recorded for cases.

After recruitment, the mother and the fetus were monitored as per standard clinical protocol, according to the severity of the growth restriction: Doppler velocimetry of umbilical artery, middle cerebral artery, ductus venosus, and computerised CTG were carried out. Similarly, the standard clinical protocol was used for timing of delivery decision. In all cases the acid-base balance was determined at birth on the umbilical artery.

After obtaining signed informed consent, patients were monitored by ta-fECG on a daily basis until delivery. The length of each recording was 37 minutes on average [interquartile range (IQR) 29–55]. For the purposes of the present study the nearest available ta-fECG monitoring to the delivery was used and Doppler velocimetry parameters were obtained within 48 hours of the ta-fECG.

Women with an uneventful pregnancy were recruited at our low-risk antenatal clinic and followed until delivery. In these cases a single ta-fECG recording was performed. A Doppler velocimetry evaluation was not performed in this group.

All women provided written informed consent prior to the ta-fECG monitoring. The study was approved by the Ethics Committee of the ICP-Istituti Clinici di Perfezionamento, Milan, Italy.

Clinical definitions

The diagnosis of IUGR was defined by an abdominal circumference ≤5th percentile for gestational age in fetuses normally developed at the time of mid-gestation ultrasound scan. Women were considered to have an uneventful pregnancy if they did not experience any obstetrical, medical or surgical complication of pregnancy, and delivered at term (≥37 weeks of gestation), without complications, a neonate whose birthweight was appropriate for gestational age (AGA) between the 10 and 90th percentile for gestational age.[9]

Trans-abdominal fetal electrocardiogram

The fHR recordings were performed with a Monica AN24 fetal ECG monitor (Monica Healthcare, Nottingham, UK). Extracted fECG complexes were used to calculate the R wave to R wave (RR) pulse intervals with an accuracy of approximately 1 millisecond (ms).

Doppler velocimetry

Pulse-wave and colour Doppler ultrasound examination of the uterine arteries, umbilical arteries, middle cerebral

artery, and ductus venosus was performed. Uterine artery Doppler velocimetry was defined as abnormal if the mean between the right and left PI was above the 95th percentile for gestational age.[10] Umbilical artery Doppler velocimetry was defined as abnormal if the PI was above the 95th percentile for gestational age[11] or for the absent or reversed end-diastolic velocities.[12] Brain sparing effect was defined as middle cerebral artery PI < 5th percentile.[11]

PRSA analysis

The PRSA computation is based on four consecutive steps. The first step assumes the definition of the anchor points (fRR intervals). An 'anchor point' is any of the fRR intervals that locally increases (for DC computation) or decreases (for AC computation). In practice, each fRR interval at time 't' that satisfies the following criterion is inserted into the decelerations anchor point list (the inequality must be reversed for accelerations):

$$\frac{1}{T}\sum_{i=0}^{T-1}\mathrm{fRR}(t+i) > \frac{1}{T}\sum_{i=0}^{T-1}\mathrm{fRR}(t-i)$$

A window of length $2L$ is centred on each anchor point, where L determines the extension of the PRSA series on each side. Next, the PRSA series is computed averaging the segments of fRR signal, identified by each of the windows. As anchor point lists are different, two separate PRSA series are computed for accelerations and decelerations.

Finally, acceleration and deceleration capacities of fHR are computed on each of the PRSA series:

$$\mathrm{DC\ (or\ AC)} = \frac{1}{2s}\sum_{i=1}^{s}\mathrm{PRSA}\,(L+i) - \frac{1}{2s}\sum_{i=1}^{s-1}(L-i)$$

The parameter 'T' sets the number of points of the low-pass moving average filter employed before the detection of anchor points in the PRSA computation, and acts as an upper frequency limit for the periodicities that can be detected; e.g. at $T = 1$ no filter is applied, whereas higher T values enhance low-frequency oscillations hidden in the signal. Similarly, the parameter 's' selects the oscillations in the PRSA series that have the greatest effect on AC and DC computation. From our previous study we found that $T = 9$ best differentiates IUGR fetuses from controls when $s = T$[7]. Such a value corresponds to nine fRR intervals before each anchor point, and other nine after it. For an exhaustive description of the PRSA technique, please refer to Bauer et al.[5]

PRSA analysis of the ta-fECG was performed off-line and on the entire length of the recordings. The series of RR intervals were obtained from the trans-abdominal data, and a simple preprocessing was performed. Fetal RR intervals > 1500 ms (corresponding to 40 beats/minute) were marked as artefacts and substituted with an equivalent number of beats (determined by dividing the length of each artefact by the median of the 20 nearby fRR samples). By design, these reconstructed points were not selected as anchor points in the PRSA computation; however, they contributed to the selection of nearby anchor points. Additionally, each fRR interval that exceeded the preceding one by more than 20% was excluded from the anchor point lists.

Statistical analysis

A Kolmogorov–Smirnov test and Shapiro–Wilk test and visual plot inspection were used to assess the normality of the distributions. Data are presented as means and standard deviations (SDs), as medians and interquartile ranges, or as absolute values and percentages. Parametric tests were computed to compare the means of continuous variables between two (t-test) or more groups (one-way ANOVA followed by *post-hoc* Bonferroni analysis for multiple group comparisons). Mann–Whitney U or Kruskal–Wallis tests were used as non-parametric alternatives. Proportions were compared using a chi-square or Fisher's exact test, as appropriate.

Logistic regression models were used to determine the association between the IUGR outcome and either AC or DC at T9, adjusting for gestational age at monitoring. The same approach was adopted to determine the association between IUGR with and without brain sparing and either AC or DC at T9. Linear regression was used to study the relation between AC or DC at T9 (dependent variables for which the hypothesis of normality had not been rejected) and Doppler velocimetry parameters, again adjusting for gestational age at monitoring. Linear and quadratic regression curves were used to summarise graphically the values of AC-T9 and DC-T9 in control fetuses. Quadratic curves fitted these values better than linear curves (both in terms of Akaike and Bayesian information criteria) and were thus used to represent them graphically.

Statistical analyses were performed with SPSS, version 21 (IBM Corp, Armonk, NY, USA), and STATA 11.2 (StataCorp, College Station, TX, USA).

Results

Demographic and clinical characteristics of the study population

In all, 145 women were recruited: 66 pregnancies with IUGR and 79 uncomplicated pregnancies. The demographic and clinical characteristics of the study population are shown in Table 1. No significant differences were observed between cases and controls as regards age, body

Table 1. Demographic and clinical characteristics of the study population. Values are expressed as mean (standard deviation), median (interquartile range) or number (percent)

	IUGR (n = 66)	Uncomplicated pregnancies (n = 79)	P
Age (years)	33.0 (5.1)	33.6 (5.4)	0.5
Nulliparous (yes)	45 (52.3%)	41 (47.7%)	0.6
BMI (kg/m^2)	22.4 (3.8)	22.8 (3.9)	0.8
Gestational age at monitoring (weeks)	33.9 (3.8)	34.5 (3.3)	0.3
Gestational age at birth (weeks)	35.1 (3.3)	39.5 (1.1)	<0.0001
Time interval between monitoring and birth (days)	2 (0; 5)	35 (18; 58)	<0.0001
Birthweight (g)	1860 (604)	3393 (387)	<0.0001

BMI, body mass index; g, grams.

mass index or parity. All uncomplicated pregnancies delivered at term and the neonates had a birthweight appropriate for gestational age (Table 1). Thus, as per study design, the time interval between monitoring and birth was higher for uncomplicated pregnancies (Table 1).

Doppler velocimetry characteristics and neonatal outcome in IUGR differed according to gestational age at diagnosis, and are reported in Table 2.

Analysis of average acceleration and deceleration capacity in relation to Doppler characteristics of intrauterine growth-restricted fetuses

At logistic regression, the AC-T9 and DC-T9 were significantly lower in IUGR fetuses than in controls after adjusting for gestational age at monitoring: for AC-T9, odds ratio (OR) = 2.1 [95% confidence interval (CI) 1.5–3.0, $P < 0.001$] and for DC-T9, OR = 0.5 (95% CI 0.36–0.68, $P < 0.001$).

Multivariate linear regression, including gestational age at ta-fECG recording, and Doppler velocimetry parameters (uterine, umbilical and middle cerebral arteries), showed a significant association in IUGR fetuses between the middle cerebral artery PI and AC-T9 and DC-T9: for AC-T9, the coefficient = –0.867 ($P = 0.01$); and for DC-T9, the coefficient = 1.016 ($P = 0.005$), respectively. For other Doppler variables the association with AC-T9 and DC-T9 did not reach statistical significance: the uterine arteries mean PI for AC-T9, the coefficient = –0.445 ($P = 0.2$) and for DC-T9, the coefficient = 0.601, $P = 0.1$; and the umbilical artery PI for AC-T9, the coefficient = 0.207 ($P = 0.3$) and for DC-T9, the coefficient = −0.250 ($P = 0.3$), respectively. These findings were also confirmed by linear regression models based on a single Doppler variable adjusted for gestational age at recording: middle cerebral artery PI: for AC-T9, the coefficient = −0.896 ($P = 0.006$) and for DC-T9, the coefficient = 1.060 ($P = 0.003$); uterine arteries mean PI: for AC-T9, the coefficient = −0.276 ($P = 0.4$) and for DC-T9, the coefficient = 0.407 ($P = 0.3$); and umbilical artery PI: for

Table 2. Clinical and Doppler velocimetry characteristics and neonatal outcome of pregnancies with IUGR according to gestational age at diagnosis. The values are expressed as mean (SD) or number (%). Perinatal complications: neonatal death, intraventricular haemorrhage, periventricular leucomalacia, necrotising enterocolitis, respiratory distress syndrome, and bronco-pulmonary dysplasia

	IUGR ≥ 25 to < 30 weeks (n = 7)	IUGR ≥ 30 to < 34 weeks (n = 15)	IUGR ≥ 34 to < 37 weeks (n = 28)	IUGR ≥ 37 weeks (n = 16)
GA at admission (weeks)	26.5 (1.9)	30.6 (2.3)	34.3 (2.3)	37.3 (1.4)
Uterine arteries mean PI	1.56 (0.48)	1.19 (0.63)	0.93 (0.30)	0.87 (0.36)
Umbilical artery PI	2.59 (1.86)	1.45 (0.55)	1.04 (0.21)	0.99 (0.31)
Umbilical artery, ARED	4 (57%)	2 (13%)	0	0
Middle cerebral artery PI < 5th percentile	7 (100%) (n = 7)	7 (47%) (n = 15)	6 (27%) (n = 22)	5 (38%) (n = 13)
Ductus venosus PI > 2 SD	4	1*	0	0
AC-T9	−2.09 (0.51)	−3.18 (0.79)	−3.70 (1.22)	−3.65 (1.09)
DC-T9	2.16 (0.62)	3.41 (0.84)	3.97 (1.34)	3.86 (1.18)
GA at delivery (weeks)	28.9 (2.8)	32.7 (1.9)	36.3 (1.2)	38.1 (0.9)
Birthweight < 10th percentile	5 (71%)	11 (73%)	21 (75%)	14 (85%)
NICU admission	7 (100%)	15 (100%)	11 (39%)	3 (19%)
Perinatal complications	7 (100%)**	9 (60%)	1 (4%)	1 (6%)

ARED, absent or reverse end diastolic flow; GA, gestational age; NICU, neonatal intensive care unit; PI, pulsatility index; SD, standard deviation.
*Missing data, n = 4.
**Includes one neonatal death.

AC-T9 the coefficient = −0.276 (P = 0.2) and for DC-T9 the coefficient = −0.324 (P = 0.2), respectively.

Figure 1 represents the AC-T9 and DC-T9 values of IUGR fetuses with and without brain sparing and controls. The IUGR fetuses with brain sparing had significantly lower AC-T9 and DC-T9 than controls at all gestational age epochs, and the association was highly significant: for AC-T9, OR = 4.4 (95% CI 2.3–8.4), $P < 0.0001$; for DC-T9, OR = 0.25 (95% CI 0.1–0.5), $P < 0.0001$, Figure 2a). IUGR fetuses without brain sparing had also lower AC-T9 and DC-T9 than controls [for AC-T9, OR = 1.9 (95% CI 1.2–2.9), P = 0.005; for DC-T9, OR = 0.6 (95% CI 0.4–0.8), P = 0.005; Figure 2b], but AC-T9 and DC-T9 values were higher for IUGR with brain sparing than for IUGR without brain sparing [for AC-T9, OR = 1.8 (95% CI 0.97–3.4), P = 0.06; for DC-T9, OR = 0.5 (95% CI 0.3–0.98), P = 0.04]. The statistical significance was reached for DC-T9 and not for AC-T9, most likely due to small sample size. When observing the distribution of IUGRs without brain sparing there appear to be two distinct population sub-groups: those with lower AC-T9 and DC-T9 than controls, and those overlapping the controls (Figure 2b).

Discussion

Main findings

This is the first study that has evaluated the association between AC and DC in IUGR fetuses and middle cerebral artery PI. We found a significant association between acceleration and deceleration capacity (computed at $s = T = 9$) of the fetal heart and middle cerebral artery PI, and no significant association with uterine or umbilical arteries Doppler velocimetry parameters. In addition, values of AC-T9 and DC-T9 were lower in IUGR with brain sparing than in IUGR without brain sparing.

Strengths and limitations

This study has several strengths in relation to the methods of signal registration (ta-fECG), and signal analysis (PRSA method). We applied ta-fECG, which provided non-invasive, passive and long recordings of fRR intervals that are essential for an accurate analysis of fHRV. In fact, the evaluation of the ANS homeostasis requires 'true' beat-to-beat information. However, the analysis of fECG recorded transabdominally is challenging due to the signal-to-noise ratio and fragmentation. In this study, we overcame this limitation by adopting the PRSA method on fECG, whereas the application of PRSA method on the standard CTG Doppler signal might have some intrinsic limitations.

The main limitation of this study is the small sample size that might have masked the identification of more subtle differences. Another limitation is that we did not have the Doppler evaluation of the AGA fetuses, although one would imagine that in fetuses with appropriate growth and from uneventful pregnancies those parameters were within normal ranges. Certainly, it would be interesting to evaluate the correlation between AC and DC in IUGR fetuses and acid-base biomarkers at birth. In our study this was not feasible as not all recordings were performed on the day of the delivery and close to labour. Moreover, such an evaluation would be difficult taking into consideration that some women delivered vaginally and others by elective cesarean section.

Last, but not least is the definition of IUGR that we adopted. We defined IUGR as abdominal circumference < 5th percentile in the presence of normal growth at the mid-gestational scan. This definition might have some limitations, especially near term, when it is difficult to differentiate constitutionally small fetuses from those that are growth-restricted. Thus, some of IUGR fetuses included in the analysis may have been constitutionally small. On the other hand, a stricter definition of IUGR, such as

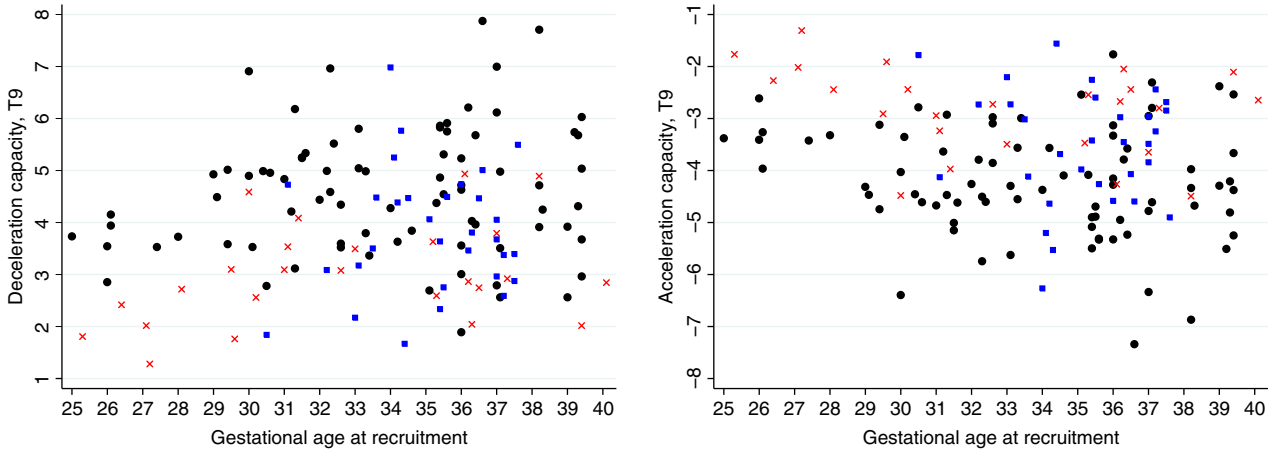

Figure 1. Deceleration capacity T9 (left panel) and acceleration capacity T9 (right panel) values observed in IUGR fetuses with brain sparing (red crosses) and without brain sparing (blue points) and control fetuses (black points). Note that acceleration capacity is a negative value and expresses time in milliseconds. GA, gestational age.

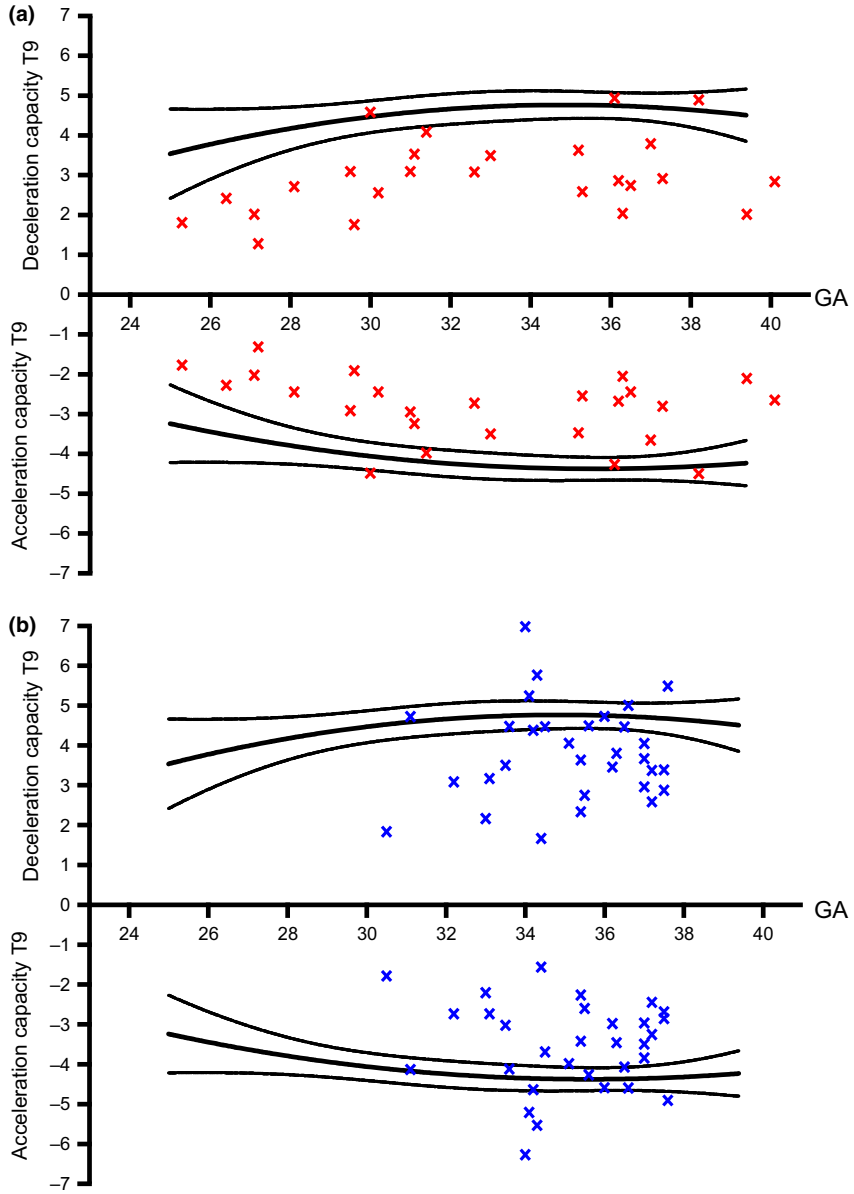

Figure 2. Deceleration capacity T9 (upper panel) and acceleration capacity T9 (lower panel) for control fetuses (bold line: quadratic mean fitted values; dashed lines: 95% confidence intervals). (a) Fetuses with intrauterine growth restriction and brain sparing (red crosses). (b) Fetuses with intrauterine growth restriction without brain sparing (blue crosses). Note that acceleration capacity is a negative value and expresses time in milliseconds. GA, gestational age.

abdominal circumference <5th percentile with increased umbilical artery Doppler PI, would have excluded from the analysis those fetuses suffering from growth restriction in the absence of umbilical artery Doppler modification. Thus, the findings from this study have a solid grounding and raise questions for future clinical studies.

Interpretation

In fetuses exposed to chronic hypoxia several mechanisms of adaptation are activated, some of them mediated by the presence of acidaemia. One of the compensatory mechanisms is the redistribution of the blood flow with an increased flow to vital organs at the expense of other body parts.[13] The redistribution of blood flow to vital organs (brain, heart and adrenal glands) is mediated by neuro-humoral mechanisms.[14] The cerebral vasodilation is thought to be due to the local effects of hypoxia on cerebral blood vessels,[13,15] with or without hypercapnia.[16] Studies on animal models confirmed that this adaptation to hypoxia is determined by aortic chemoreceptors or carotid baro- and chemoreceptors.[17,18] The latter,

together with the somatosensory stimulation, are also responsible for the neural control of the cardiovascular system.[19] Thus, it is not surprising that the acceleration and deceleration capacities of the fetal heart, as measured by PRSA, are associated with middle cerebral artery PI, which is a consistent index of brain sparing adaptation. Indeed, the underlying controlling mechanisms in both are responsive to mild/moderate hypoxaemia.[15] Conversely, high values of umbilical artery Doppler parameters are not correlated with poor oxygenation *per se,* unless associated with ominous changes of the fetal heart rate[13,20] or in the presence of extreme alterations of the umbilical artery flow, such as absence of end diastolic flow.[21,22]

The understanding of the temporal sequence of changes of biophysical parameters in IUGR fetuses is important for monitoring and timing of delivery criteria. The cascade of the events has been widely studied by several groups with regards to early severe IUGR fetuses and it is now known that in this subset of fetuses, abnormal umbilical artery blood flow velocity occurs prior to middle cerebral artery low PI, and fHRV reduction and long before the occurrence of fetal distress.[1] At the other end of this sequence, decreased fHRV and fHR decelerations have been found to be associated with hypoxaemia at caesarean section and at cordocentesis.[20] More recent studies addressed a similar problem in late IUGR.[23,24] Few data are available as regards direct measures of oxygenation in these fetuses and thus should be extrapolated from late IUGRs reported in previous studies.[20] However, a consistent finding underlines that the same mechanism of brain sparing observed in early severe IUGR fetuses is at work in late IUGRs.[23,24]

In our cohort of IUGR fetuses from 25 weeks of gestation to term, decreased acceleration and deceleration capacities were significantly associated with changes in the middle cerebral artery. This could be interpreted as an early event in the cascade of biophysical changes observed at any gestational age, the result of changes that induce both the brain sparing adaptation and ANS modification influencing fHRV. However, this study did not address the issue of longitudinal changes of the biophysical parameters in IUGR fetuses and did not relate them to the perinatal outcome. Appropriately designed studies should be performed to confirm this hypothesis.

Overall, AC and DC were also lower in IUGR fetuses without brain sparing than in controls, but the association was not as significant as for IUGR with brain sparing. However, it would seem that there is a subgroup of IUGR fetuses without brain sparing with lower AC and DC than controls, and a subgroup with AC and DC values overlapping those of the controls. We were not able to find a clear explanation for this observation. A hypothesis might be that in late-preterm and term IUGRs there is a subgroup of 'small' babies who suffer growth restriction in the presence of 'normal waveforms' at feto-placental Doppler velocimetry evaluation including the middle cerebral artery. It remains to be elucidated whether the AC/DC are more sensitive measures than Doppler velocimetry evaluation of fetal districts in identifying those fetuses and differentiating them from constitutionally small fetuses.

Conclusion

Our study observed for the first time a significant decrease of acceleration and deceleration capacity at PRSA analysis of fetal heart rate variability in association with changes in middle cerebral artery PI in IUGR fetuses from 25 weeks of gestation to term, but not changes to uterine and umbilical artery PI.

The PRSA method allows separate characterization of acceleration- and deceleration-related modulations of fHRV, differing from short-term variation (STV), which incorporates both components. We and others[25,26] have already demonstrated that AC and DC are superior to STV in discriminating IUGR from AGA fetuses. The potential advantages of the PRSA method over, or in addition to, standard methods of monitoring such as Doppler velocimetry and/or computerised CTG, and its efficacy in clinical practice has still to be explored.

Disclosure of interests

Full disclosure of interests available to view online as supporting information.

Contribution to authorship

T.S. contributed to the study design, the fHR and statistical analysis, and to writing the article. D.C. contributed to the maintenance of the database and the fHR analysis. L.M. contributed to statistical analysis and to writing the article. R.S. and M.W.R. contributed to fHR analysis and to writing the article. M.L.M, A.B. and E.F. contributed to the interpretation of the data and to writing the article.

Details of ethics approval

This study was approved by ethics committee 'Azienda Ospedaliera – Istituti Clinici di Perfezionamento – Milano' on the 1 August 2009 (reference FETAB-ECG).

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