Does heart rate variability reflect the systemic inflammatory response in a fetal sheep model of lipopolysaccharide-induced sepsis?

Lucien D Durosier¹, Christophe L Herry², Marina Cortes⁶, Mingju Cao¹, Patrick Burns³, André Desrochers³, Gilles Fecteau³, Andrew J E Seely^{2,4,5}, and Martin G Frasch^{1,6}

¹Dept. of OBGYN and Dept. of Neurosciences, CHU Ste-Justine Research Centre, l'Université de Montréal, Montréal, QC, Canada

²Clinical Epidemiology Program, Ottawa Hospital Research Institute, Ottawa, Canada

³Clinical Sciences, CHUV, l'Université de Montréal, St-Hyacinthe, QC, Canada

⁴Department of Molecular and Cellular Medicine, University of Ottawa, Ottawa, Canada

⁵Divisions of Thoracic Surgery & Critical Care Medicine, The Ottawa Hospital, Ottawa, Canada

⁶Centre de recherche en reproduction animale, l'Université de Montréal, St-Hyacinthe, QC, Canada

Abstract

Fetal inflammatory response occurs during chorioamnionitis, a frequent and often subclinical inflammation associated with increased risk for brain injury and life-lasting neurologic deficits. No means of early detection exist. We hypothesized that systemic fetal inflammation without septic shock will be reflected in alterations of fetal heart rate (FHR) variability (fHRV) distinguishing baseline versus inflammatory response states.

In chronically instrumented near-term fetal sheep ($n = 24$), we induced an inflammatory response with lipopolysaccharide (LPS) injected intravenously ($n = 14$). Ten additional fetuses served as controls. We measured fetal plasma inflammatory cytokine IL-6 at baseline, 1, 3, 6, 24 and 48 h. 44 fHRV measures were determined continuously every 5 min using continuous individualized multi-organ variability analysis (CIMVA). CIMVA creates an fHRV measures matrix across five signal-analytical domains, thus describing complementary properties of fHRV. Using principal component analysis (PCA), a widely used technique for dimensionality reduction, we derived and quantitatively compared the CIMVA fHRV PCA signatures of inflammatory response in LPS and control groups.

In the LPS group, IL-6 peaked at 3 h. In parallel, PCA-derived fHRV composite measures revealed a significant difference between LPS and control group at different time points. For the LPS group, a sharp increase compared to baseline levels was observed between 3 h and 6 h, and then abating to baseline levels, thus tracking closely the IL-6 inflammatory profile. This pattern was not observed in the control group. We also show that a preselection of fHRV measures prior to the

PCA can potentially increase the difference between LPS and control groups, as early as 1 h post LPS injection.

We propose a fHRV composite measure that correlates well with levels of inflammation and tracks well its temporal profile. Our results highlight the potential role of HRV to study and monitor the inflammatory response non-invasively over time.

Keywords

monitoring; HRV; fetus; LPS; principal component analysis; inflammation; bioinformatics

Introduction

The main manifestation of pathologic inflammation in the fetoplacental unit, chorioamnionitis, affects 20% of term pregnancies and up to 60% of preterm pregnancies; notably, it is often an occult finding (Gotsch *et al* 2007). Both symptomatic and asymptomatic chorioamnionitis are associated with a ~9-fold increased risk of cerebral palsy (Grether and Nelson 1997). Even asymptomatic inflammation may inhibit placental angiogenesis and thus modulate the course of the pregnancy (Garnier *et al* 2008). Thus, a significant number of fetuses are exposed to variable degrees of inflammation that may impact their brain development. Current methods to diagnose fetal compromise due to an infectious or inflammatory condition are inadequate (Garite 2001). Hence, a need exists to identify early signs of the fetus at risk of adverse outcome and intervene therapeutically (Garite 2001).

Via the vagus nerve, the cholinergic anti-inflammatory pathway (CAP) provides negative feedback on systemic levels of inflammatory cytokines (Tracey 2009). This rapid homeokinetic control of the inflammatory milieu is reflected in subtle alterations of fetal heart rate (FHR) variability (fHRV) (Tracey 2009). Variations in fetal vagal activity can be measured non-invasively by monitoring the beat-to-beat fHRV (Durosier *et al* 2014). Importantly, derived from fetal ECG sampled at 1000 Hz, such beat-to-beat fHRV is more precise than the time-averaged fHRV used currently clinically, for which a sampling rate of less than 4 Hz is used (Durosier *et al* 2014). Perinatal studies show that beat-to-beat fHRV has potential as a non-invasive, continuous, sensitive and specific measure of the fetal inflammatory response (Fairchild and O'Shea 2010). HRV measures can be derived from various signal-analytical domains. RMSSD (root mean square of standard deviation), a measure of short-term HRV, certain complexity and other signal domain measures reflecting short term time scale fHRV may serve as indicators of this complex vagal modulation of inflammatory activity.

Lipopolysaccharide (LPS)-induced inflammation in fetal sheep is a well-established model of the human fetal inflammatory response to sepsis (Rees and Inder 2005). However, whether this process can be tracked via fHRV monitoring has not been studied. We hypothesized that distinct patterns of fHRV measures will correlate to LPS-triggered rise in pro-inflammatory cytokines.

Methods

Animal care followed the guidelines of the Canadian Council on Animal Care and the approval by the University of Montreal Council on Animal Care (protocol #10-Rech-1560).

Anesthesia and surgical procedure

Details of the procedure are provided as supplementary material (stacks.iop.org/PM/36/2089/mmedia). Briefly, we instrumented 24 pregnant time-dated ewes at 126 d of gestation (dGA, ~0.86 gestation) with arterial, venous and amniotic catheters and ECG electrodes.

Antibiotics were administered to the mother intravenously (Trimethoprim sulfadoxine 5 mg kg⁻¹) as well as to the fetus intravenously and into the amniotic cavity (ampicillin 250 mg). The exteriorized catheters were secured to the back of the ewe in a plastic pouch. For the duration of the experiment the ewe was returned to the metabolic cage, where she could stand, lie and eat *ad libitum* while we monitored the non-anesthetized fetus without sedating the mother. During postoperative recovery antibiotic administration was continued for 3 d. Arterial blood was sampled for evaluation of maternal and fetal condition and catheters were flushed with heparinized saline to maintain patency.

Experimental protocol

Postoperatively, all animals were allowed 3 d to recover before starting the experiments. On these 3 d, at 9:00 am 3 mL arterial plasma samples were taken for blood gases and cytokine analysis. Each experiment commenced at 9:00 am on post-operative day 4 with a 1 h baseline measurement followed by the respective intervention as outlined below. FHR and arterial blood pressure was monitored continuously (CED, Cambridge, UK, and NeuroLog, Digitimer, Hertfordshire, UK). Blood and amniotic fluid samples (3 mL) were taken for arterial blood gases, lactate, glucose and base excess (in plasma, ABL800Flex, Radiometer) and cytokines (in plasma and amniotic fluid) at the time points 0 (baseline), +1 (i.e. 1 h after LPS administration), +3, +6, +24, +48 and +54 h (i.e. before sacrifice at experimental day 3). For the cytokine analysis, plasma was spun at 4 °C (4 min, 4000 g force, Eppendorf 5804 R, Mississauga, ON), decanted and stored at -80 °C for subsequent ELISAs. After the +54 h (experimental day 3) sampling, the animals were sacrificed. Fetal growth was assessed by body, brain, liver and maternal weights.

Fourteen fetuses received LPS (400 ng/fetus/day) (Sigma L5293, from *Escherichia coli* O111:B4, readymade solution containing 1 mg mL⁻¹ of LPS) intravenously on days 1 and 2 at 10.00 am to mimic high levels of endotoxin in fetal circulation over several days as it may occur in chorioamnionitis. Ten fetuses were used as controls receiving an equivalent volume of NaCl 0.9% in lieu of LPS.

Cardiovascular analysis

Mean ABP (mABP) and FHR were calculated for each animal, at each time point (baseline, 1 h, 3 h, 6 h, 24 h, 48 h and 54 h), as an average of the artifact-free 30 preceding minutes (60 preceding minutes for the baseline) using Spike 2 (Version 7.13, CED, Cambridge, UK).

FHRV analysis

The continuous individualized multiorgan variability analysis (CIMVA; see table S1 of the supplementary material: stacks.iop.org/PM/36/2089/mmedia) server platform was used to develop comprehensive continuous fHRV measures analysis. The complete fetal ECG was uploaded onto the CIMVA server to generate continuous fHRV measures, along with measures of data quality for every interval evaluated. By offering a standardized, comprehensive and validated linear and nonlinear fHRV analysis software platform, CIMVA and principal component analysis (PCA) approach allowed us to assess the temporal relation of multiple fHRV measures to manipulations of the fetal innate immune system's response to endotoxin and the potential clinical value of linear and nonlinear fHRV measures for monitoring fetal inflammatory response.

Each fHRV measure was normalized with respect to the mean and range over each animal's individual baseline, in order to track variability changes and the impact of LPS injection and resulting inflammation over time. A PCA was carried out on fHRV measures for each animal from the LPS group. Only the top PCA components accounting for more than 90% of the variance in the data were kept and the corresponding PCA coefficients were averaged over the LPS group. Finally, using the averaged PCA coefficients, the decomposition was applied to animals from both groups and the sum of resulting components yielded animal-specific fHRV time profiles. This fHRV composite measure was also compared to heart rate alone in order to highlight the added value and potential of using multiple fHRV measures as an fHRV signature of inflammation.

Cytokine analyses

Cytokine concentrations (IL-6, TNF- α) in plasma were determined by using an ovine-specific sandwich ELISA. Details are provided as supplementary material (stacks.iop.org/PM/36/2089/mmedia).

Statistical analysis

Generalized estimating equations (GEE) modeling was used to assess the effects of LPS while accounting for repeated measurements on fetal blood gases and acid-base status, plasma cytokines (IL-6 and TNF- α) and cardiovascular responses. We used a linear scale response model with time and LPS as predicting factors to assess their interactions using maximum likelihood estimate and Type III analysis with Wald Chi-square statistics. SPSS Version 21 was used for these analyses (IBM SPSS Statistics, IBM Corporation, Armonk, NY). The median change of fHRV (across fHRV measures) at each time point was compared across groups using a Wilcoxon rank-sum test on the medians. We corrected for multiple comparisons (7 time points) using the Benjamini and Yekutieli False Discovery Rate procedure (Ratcliffe and Shults 2008). We also assessed the global statistical significance of the difference in medians between time point and groups using a quasi-least squares approach within the GEE framework with a Markov Correlation structure and normal distribution assumption. We used Ratcliffe and Shults' Matlab implementation (Ratcliffe and Shults 2008). All results are presented as mean $\pm$ SD. Statistical significance was assumed for $P < 0.05$.

Results

Cohorts' characteristics

Maternal weight averaged 77 ± 10 kg. Maternal venous blood gases, pH and lactate did not significantly change during the experiments and were within physiological range throughout the experiment for both groups. Averaged over all measurement time points, they were: pO_2 53.7 ± 6.2 mmHg; pCO_2 40.9 ± 1.5 mmHg, pH 7.44 ± 0.01 ; lactate 0.66 ± 0.15 mmol l^{-1} .

Fetal body weights averaged was 3.6 ± 0.8 kg. Gestational age at time of the experimental day 1 averaged $130 d \pm 0.7$ dGA (term 145 dGA). In the control group 4/7 fetuses were male and 5/7 singletons. In the LPS group 4/10 fetuses were male and 2/10 singletons.

Clinical-chemical data

Basal fetal arterial blood gases, pH (7.37 ± 0.04), BE (3.3 ± 2.3 mmol l^{-1}) and lactate (1.5 ± 0.9 mmol l^{-1}) were within physiological range during the baseline in both groups (figure 1). We found significant time-LPS interactions for pH ($P = 0.03$), pO_2 , pCO_2 , lactate and BE (all $P < 0.001$) (figure 1(A)).

Cardiovascular analysis

We found time-LPS interactions for mABP and FHR responses ($P = 0.015$ and $P < 0.001$, respectively, figure 1(B)). This was significant for FHR at 6 h ($P = 0.008$).

Plasma cytokines response to LPS

We detected time-LPS interaction for IL-6 ($P < 0.001$) and for TNF- α ($P = 0.002$) (figure 1(C)). The LPS group showed a peak of IL-6 at 3 h.

Analysis of CIMVA fHRV signature of fetal inflammatory response: PCA

The evolution over time of the averaged PCA-derived composite fHRV measure for both LPS and control groups was derived, using 44 fHRV measures and retaining 6 PCA components to generate the composite measure, which accounted for 90% of the variance in the data. Qualitatively, the discrimination between LPS-injected animals and control animals occurs between 2 h and 3 h post injection, which is consistent with the IL-6 peak at 3 h following the LPS injection. A statistical comparison of the change in median value at 1 h, 3 h, 6 h, 24 h and 48 h and across groups is shown in table 2 for the composite fHRV and FHR alone, respectively. The time and group effects are statistically significant at the 0.05 level for the composite fHRV measure. Only the group effect is significant for FHR. Finally, the adjusted p -values comparing LPS-injected animals with the control group at baseline, 1 h, 3 h, 6 h, 24 h and 48 h are reported in table 3.

Pre-selection of fHRV features further improves the detection of inflammation

Within the context of early detection of fetal inflammation, we can identify a subset within the 44 fHRV measures used in the previous section that might better discriminate between LPS and control groups. As an example, we selected two time periods (2 h30 to 3 h and 5 h30 to 6 h), corresponding to the observed peak response of IL-6 in fetal plasma to LPS

exposure at 3 h and 6 h (see figure 1(C)) and averaged the fHRV measures at each period. We then used a minimum redundancy maximum relevance (MRMR) feature selection technique based on mutual information (Duncombe *et al* 2010) to rank the fHRV measures with respect to the classification of LPS versus control animals. We chose the fHRV measures that were both present in the top 10 ranked fHRV measures at both time periods, which resulted in only 4 fHRV measures, notably with FHR absent, and 2 PCA components (see table S2 at stacks.iop.org/PM/36/2089/mmedia). The time profile plots are shown in figure 2. The confidence intervals on fHRV composite profiles are tighter than in the profiles derived from the 44 fHRV measures (not shown), for both groups. This in turn translates into lower adjusted *p*-values and statistical significance as early as 1 h post LPS injection, when comparing the two experimental groups, as shown in table 3. The time and group effects are shown in table 2 (with heading ‘fHRV composite 2’). For comparison, the average fetal heart rate over time for both groups is shown in figure 3.

The increase in variability is confirmed by the time profile plots of each individual measure part of the composite measure of variability, provided in figures S1 through S4 of the supplementary material (stacks.iop.org/PM/36/2089/mmedia). Briefly, they show some degree of discrimination between the two groups, although not statistically significant at most time points, as can be seen qualitatively with the shaded confidence intervals. The time irreversibility asymmetry index (AsymI) shows the greater discrimination, which may potentially become significant with a greater population.

Discussion

We demonstrated that multidimensional fHRV analysis can track the temporal profile of fetal inflammation and discriminate between normal and inflammatory processes across time. Our findings may eventually lead to design of bedside fetal monitors for early detection of subclinical inflammation.

Our experimental cohort’s morphometric, arterial blood gases, acid-base status and cardiovascular characteristics were within physiological range and representative for late-gestation fetal sheep as a model of human fetal development near term (Frasch *et al* 2007). The effect of the chosen LPS dose on the arterial blood gases, acid-base status and cardiovascular responses is compatible with a septicemia (mild compensated metabolic acidemia and hypoxia) evidenced by a transient rise of IL-6 at 3 h without shock and without cardiovascular decompensation. The cytokine rise was accompanied by a slight drop of mABP and a rise in FHR. This explains why mean FHR was selected as one of the fHRV measures profiling the inflammation. However, as we discuss below, FHR alone does not suffice to predict the inflammation early or track it over time.

The selective rise of IL-6, but not TNF- α is in line with literature at this developmental stage (Duncombe *et al* 2010, Chan *et al* 2013). Here we report that despite the lack of zero-moment change in TNF- α concentration over time, LPS caused an overall decrease in TNF- α concentration. This is a novel observation, since the clinical studies reporting no change in TNF- α in perinatal inflammation or labour usually sample only once from the cord blood. Our finding is in line with the general notion that sepsis may suppress hormone secretion

variability (Rassias *et al* 2005, Scheff *et al* 2013). Further studies are needed to test whether a similar pattern applies to other cytokines such as IL-8 and IL-10.

At 24 h, when the second LPS dose was administered, we observed the well-known immunological tolerance effect with lack of significant renewed rise in IL-6. Furthermore, there was still a significant difference in fHRV between the LPS and control group at both 24 h and 48 h, although the difference was far less than at the peak of inflammation (3 h).

Fairchild *et al* showed how pathogen-induced sepsis and inflammatory response in adult mice impact the higher order heart rate characteristics that can be derived from HRV (Fairchild *et al* 2011). Such heart rate characteristics can be monitored non-invasively and continuously providing insights into the activity of autonomic nervous system (ANS), the vagus nerve in particular, and its control of innate immune responses to infection via the CAP (Olofsson *et al* 2012). The authors hypothesized that HRV should increase following CAP activation in response to infection. Their findings seemingly reject the initial hypothesis and support the clinically known phenomenon of ‘suppressed HRV’. CAP has been identified in adult animals and humans (Tracey 2009). Multiple epidemiologic studies have shown that low HRV, as reflected in root mean square of the successive differences of R–R intervals of ECG (RMSSD) or high frequency (HF) band spectral power, may serve as a marker of vagal modulation of inflammatory activity in adults (Haensel *et al* 2008, von Kanel *et al* 2008). Clinico-pathologic studies indicate that loss of CAP’s inhibitory influence unleashes innate immunity, producing higher levels of pro-inflammatory mediators that exacerbate tissue damage, and decreases short-term time scale HRV (Haensel *et al* 2008). Fairchild *et al* focused on long-term time scale HRV only, while the authors’ hypothesis and the wiring of the CAP both imply that infection and sepsis will modulate vagal signaling. That is, short-term time scale HRV would increase, but not necessarily the long-term time scale HRV. Classic examples of short-term HRV are RMSSD and HF band spectral power (Task Force 1996). The relation of these HRV measures to CAP is now well documented by the above cited clinical studies, at least in adult animals and humans. RMSSD and HF band spectral power are also decreased in fetal sheep near-term following atropine blockade (Frasch *et al* 2009). This identifies such short-term fHRV measures as reflecting vagal modulations of fHRV.

Furukawa *et al* demonstrated that CAP is active in neonatal rats and its stimulation decreases hypoxic-ischemic brain damage (Furukawa *et al* 2013). We have shown in fetal sheep that atropine blockade has no effect on SDNN, standard deviation of R–R intervals of ECG, a measure of long-term time scale fluctuations in HRV (Frasch *et al* 2009). In contrast, others demonstrated an SDNN decrease in adult mice (Laude *et al* 2008). Both studies report a significant tachycardia as a result. These differences suggest that subtle, but relevant species variations may exist in the HRV time scales at which vagal activity operates. Moreover, ANS development depends on the individual *in utero* growth trajectories (Frasch *et al* 2007) and is also species-dependent with regard to sympathetic and vagal activation patterns (Frasch *et al* 2012). The very notion of sympathetic versus parasympathetic activation has been challenged, laying foundation for the concept of sympatho-vagal co-activation as evidenced in different studies, species and developmental stages (Beuchee *et al* 2012, Frasch *et al* 2012). This translates into the requirement to presume and seek for more complex signatures

of intrinsic and challenged ANS behaviour reflected in HRV than mere states of sympathetic or vagal dominance.

Thus, further integrative studies in neonatal models of sepsis are needed to elucidate the clinical observation of bradycardia and long-term time scale HRV depression during sepsis and how this phenomenon relates to CAP activity. We suggest that such studies should not seek for HRV depression *per se*, but rather for patterns of HRV represented by its multidimensional characteristics, for example as this is done by the CIMVA system we deployed in the present study. This approach permitted us to avoid limiting ourselves *a priori* to particular fHRV measures, but, rather, allowed unbiased seeking for HRV measures signatures reflective of unchallenged, baseline, and inflammation states. To achieve this goal we have deployed a PCA-driven statistical analysis (Sorani *et al* 2007, Hemphill *et al* 2011).

During completion of this manuscript Lear *et al* reported a somewhat similarly designed experiment in the same animal model exposing a considerably less mature fetal sheep (0.7 gestation) to a higher LPS dose in an 'acute-on-chronic' design to recapitulate a scenario when an acute infection exacerbates a latent, subclinical inflammation (Lear *et al* 2014). The group studied changes in the fHRV measure RMSSD in relation to the LPS-induced cardiovascular responses. In contrast to our findings with low dose, subclinical LPS exposure triggering pronounced changes in a range of complementary fHRV measures, the authors found no evidence of RMSSD changes under conditions of chronic low-dose LPS exposure over five days. The subsequent acute higher LPS dose triggered, expectedly, hypotensive responses accompanied by an initial transient RMSSD increase followed by a RMSSD decrease due to the second acute high LPS dose exposure. These findings support the general notion that fHRV is sensitive to fetal inflammation and underscore that detection of latent fetal inflammation requires more sophisticated approaches to characterization of fHRV, such as the here presented multidimensional analysis of complementary fHRV measures from different signal-analytical domains.

Our understanding of the dynamics of fHRV in human and ovine fetuses during physiologic (e.g. sleep states) and pathophysiologic (e.g. asphyxia, sepsis) conditions has evolved over the past two decades (Karin *et al* 1993, Lake *et al* 2003, Frank *et al* 2006, Fairchild and O'Shea 2010). Late gestation human fetuses exhibit nonlinear cardiac dynamics and higher vagal tone is associated with more efficient regulation of homeostasis (Groome *et al* 1999a, Groome *et al* 1999b). FHR and fHRV are regulated by a complex interplay of the parasympathetic and sympathetic nervous systems accounting for the baseline FHR as well as short-term and long-term fHRV showing linear and nonlinear properties (Frasch *et al* 2009). These fHRV properties are differentially affected by LPS-induced fetal and neonatal inflammatory response (Lake *et al* 2002, Stone *et al* 2013). Decreased fetal and neonatal HRV and transient repetitive heart rate decelerations coincide with or precede clinical signs of sepsis (Rudolph *et al* 1965, Cabal *et al* 1980, Fairchild and O'Shea 2010). This may be due to an altered vagal tone with intermittent vagal firing in the setting of a systemic fetal inflammatory response during sepsis (Fairchild and O'Shea 2010). In an experimental model of sepsis in adults, sympathetic activation by infusion of epinephrine before administration of endotoxin reduced high-frequency HRV, suggesting vagal hyporesponsiveness (Haensel *et al* 2008). Severe sepsis could induce a state of generally decreased vagal efferent firing or

responsiveness, leading to fewer normal small FHR decelerations. On the other hand, CAP activation in sepsis via vagal efferent signaling would decrease FHR and increase fHRV (Fairchild and O'Shea 2010). Based on this animal and human clinical perinatal body of evidence, we propose that developing longitudinal and comprehensive fHRV monitoring in a model of LPS-induced inflammation will allow us to build algorithms to improve early diagnosis of infection. Such monitoring would capture both linear and nonlinear fHRV properties, a strategy that has proven effective in septic adult and neonatal patients (Seely and Macklem 2004, Ahmad *et al* 2009, Fairchild *et al* 2013).

Our studies in near-term fetal sheep further suggest that continuous assessment of fHRV between and during the FHR decelerations at precision levels higher than currently deployed clinical non-stress tests can provide further insights into the dynamics of autonomic nervous system responses to acidemia due to umbilical cord compressions. Measures derived from fHRV show the potential to serve as indicators of incipient fetal acidemia as early as 60 min in advance of pH drop to less than 7.00 (Frasch *et al* 2009, Durosier *et al* 2014).

An important observation in our study is the fHRV increase seen in a set of four fHRV measures as early as 1 h post endotoxin exposure and rising rather than the expected HRV drop that is usually observed in infectious illnesses in adult humans. These four fHRV measures are nonlinear and belong to the energetic, informational and invariant signal analytical domains of HRV, rather than to the statistical domain where the measures such as RMSSD or SDNN are found and which are linear (see table S2: stacks.iop.org/PM/36/2089/mmedia). The two metrics from the energetic domain likely reflect a complex temporal organization of HRV control mechanisms, including, but probably not limited to the autonomic nervous system activity (Ivanov *et al* 1999, Meyer and Stiedl 2003, Costa *et al* 2008). The multifractal spectrum cumulant of the first order quantifies the robustness of multifractal scaling in heart rate dynamics (Ivanov *et al* 1999, Meyer and Stiedl 2003). The time irreversibility asymmetry index quantifies the degree of temporal asymmetry of a signal, i.e. how much energy is dissipated during the development of the process (Costa *et al* 2008). In time series analysis, that corresponds to the modification of the statistical properties of a signal under the operation of time reversal. Meanwhile, the two other fHRV measures comprising the PCA-derived composite measure, 'Allan factor distance from a Poisson distribution' (Teich 2000) and 'embedding scaling exponent' (Michieli *et al* 2010), represent novel measures with regard to their utility in assessing fetal pathophysiology and require further validation in experimental settings impacting HRV. Overall, these findings support our contention that multidimensional fHRV characterization may augment the predictive ability of fHRV monitoring for detecting events such as early inflammatory response. The fHRV increase, rather than decrease, may be explained not only by the fHRV measures identified as early markers of inflammation, but also by the underlying fetal physiology at this developmental stage and how it responds to an acute septic, as is the case in the present study using LPS, or aseptic inflammatory stimulus. With regard to aseptic stimulation, we observed RMSSD increase due to worsening fetal acidemia and accompanying fetal inflammatory response induced by intermittent repetitive umbilical cord occlusions at similar gestational age in fetal sheep (Prout *et al* 2010, Durosier *et al* 2014). To test the possibility that time window length in which fHRV was computed influenced the result, we increased the window length from 5 to 10 min (data not shown). We did not see

any change in our findings and the above fHRV measures continued to increase with developing fetal inflammatory response. We conclude that endotoxin exposure triggers an early, as soon as 1 h post LPS injection, fHRV increase that represents a physiological response to inflammation.

Significance and perspectives

We propose two variants of PCA-derived composite fHRV measures that seem to best characterize the inflammatory state and perform better than single fHRV measures, such as the FHR itself. The components of the initial set of HRV measures, as well as those of the reduced set of four measures highlighted in the results section, belong to different HRV domains, which suggests that such multidimensional representation of HRV reflects underlying code carrying information about neuroimmunological, and possibly intrinsic cardiac, interactions modulated by system's state. Notably, the preselection of fHRV measures prior to the PCA revealed the difference between LPS and control groups as early as 1 h post LPS injection, using measures assessing fractal and scale-invariant fHRV properties. This result is encouraging and needs to be validated on a larger dataset.

Future work will focus on more detailed delineation of the intrinsic versus ANS-modulated HRV signatures in the physiological and pathophysiological contexts. The physiological context will include linking different signal-analytical domains of HRV such as fractal variability and complexity to the underlying processes characteristic of any living organism, such as metabolism and entropy production (Seely and Macklem 2012). Carving out the pathophysiological context of HRV will provide the framework to increase the basic and clinical understanding of the fHRV signatures presented herein and drive the development of bedside algorithms for detection of fetal inflammation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) Arterial blood gas and acid-base responses to lipopolysaccharide. Blue, control group ($n = 5$); red, LPS group ($n = 10$) at baseline, 1 h, 3 h, 6 h, 24 h, 48 h and 54 h after start of the experiment. Mean $\pm$ SD. We found a significant time-LPS interactions for pH ($P = 0.03$), pO₂, pCO₂, lactate and BE (all $P < 0.001$). (B) Cardiovascular responses to lipopolysaccharide. Blue, control group ($n = 6$); red, LPS group ($n = 10$); mABP, fetal mean arterial blood pressure in mmHg; FHR, fetal heart rate at baseline in beats per minute (bpm), 1 h, 3 h, 6 h, 24 h, 48 h and 54 h after start of the experiment. Mean $\pm$ SD. We found time-LPS interaction for mABP and FHR responses ($P = 0.015$ and $P < 0.001$, respectively). This was significant for FHR at 6 h ($P = 0.008$). (C) Fetal inflammatory response to lipopolysaccharide. Blue, control group ($n = 5$); red, LPS group ($n = 10$); Mean $\pm$ SD. *, $P = 0.001$ versus control.

Figure 2.

Temporal profile of fHRV composite measure #2, averaged over LPS and control groups. Temporal profile of the averaged PCA-derived composite fHRV measure for both LPS (10 animals) and control (7 animals) groups, using 4 fHRV measures, and 2 subsequent PCA components. The fHRV composite measure is normalized with respect to each animal's baseline before being averaged across groups. Lightly shaded areas correspond to the confidence intervals around the mean. The number of animals used in the average plot may vary across time due to missing fHRV values (from periods of low quality ECG data). The time periods not shown were not available or had too many missing values. The common baseline value is indicated as a dotted line.

Figure 3.

Temporal profile of fetal heart rate, averaged over LPS and control groups. Temporal profile of the fetal heart rate for both LPS (10 animals) and control (7 animals) groups. The fetal heart rate is normalized with respect to each animal's baseline before being averaged across groups. Lightly shaded areas correspond to the confidence intervals around the mean. The number of animals used in the average plot may vary across time due to missing fHRV values (from periods of low quality ECG data). The time periods not shown were not available or had too many missing values. The common baseline value is indicated as a dotted line.

Table 1Description of heart rate variability domains^a.

Domain	Features
<i>Statistical</i>	The statistical domain consists of statistical measures (mean, standard deviation, Gaussian, and so on) describing the data distribution. It assumes the data originates from a stochastic process.
<i>Geometric</i>	The geometric domain describes the properties related to the shape of the dataset in space. This includes, in a deterministic system, grid counting, heart rate turbulence, spatial filling index and Poincaré and recurrence plots.
<i>Energetic</i>	The energetic domain describes the features related to the energy or the power of the data, such as frequency, periodicity, and irreversibility in time.
<i>Informational</i>	The informational domain describes the degree of complexity and irregularity in the elements of a time series, such as distance from periodicity or from a reference model. It includes various measures of entropy (compression, fuzzy, multiscale and so on).
<i>Invariant</i>	The invariant domain describes the properties of a system that demonstrate fractality or other attributes that do not change over either space or time. Included are scaling exponents, fluctuation analysis, and multifractal exponents.

^aDomains suggested for continuous individualized multiorgan variability analysis (CIMVA platform). After Bravi *et al* 2011.

Table 2

Statistical significance of time and group effects^a on the median change of fetal heart rate variability (fHRV) corresponding to PCA-derived fHRV composite measures (using all fHRV measures as inputs or selected measures) and heart rate, from control and LPS group (see figure 2).

fHRV composite 1					
Variable	Beta	Std. error	z value	p-value	95% CI
Time	−0.008 4	0.002 7	−3.161 2	0.001 6	[−0.013 6–0.003 2]
Group	1.501 3	0.314 2	4.778 9	1.7×10^{-6}	[0.885 6 2.117 0]
Constant	−0.522 1	0.244 2	−2.138 0	0.032 5	[−1.000 7 0.043 5]
fHRV composite 2					
Variable	Beta	Std. error	z value	p-value	95% CI
Time	−0.007 4	0.002 8	−2.619 5	0.008 8	[−0.012 9–0.001 9]
Group	1.403 2	0.176 2	7.961 8	1.7×10^{-15}	[1.057 8 1.748 6]
Constant	−0.586 5	0.147 2	−3.985 0	6.7×10^{-5}	[−0.874 9–0.298 0]
Heart rate					
Variable	Beta	Std. error	z value	p-value	95% CI
Time	−0.001 9	0.003 5	−0.538 4	0.590 3	[−0.008 8 0.005 0]
Group	1.031 4	0.192 5	5.357 8	8.4×10^{-8}	[0.654 1 1.408 7]
Constant	−0.528 4	0.179 5	−2.943 1	0.003 2	[−0.880 3–0.176 5]

^aUsing a quasi least squares method within the framework of generalized estimating equations, with a Markov correlation structure. The standard errors, 95% confidence intervals, and *p*-values are for the tests Beta = 0. Both time and group effects are statistically significant at the 0.05 level for both fHRV composite measures, only the Group effect is significant for heart rate. fHRV

Composite 1: based on 44 fHRV measures, 6 PCA components. fHRV Composite 2: based on 4 fHRV measures, 2 PCA components.

Table 3

Adjusted *p*-values from statistical comparison between LPS and control groups at each time point (using false discovery rate to account for multiple comparisons).

Adjusted <i>P</i> -values	baseline	1 h	3 h	6 h	24 h	48 h
fHRV composite 1	0.474 1	0.205 6	0.001 2 ^b	0.001 2 ^b	0.691 8	0.001 2 ^b
fHRV composite 2	0.661 1	0.020 0 ^a	0.000 8 ^b	0.001 2 ^b	0.000 8 ^b	0.011 3 ^a
Heart rate	0.661 1	0.432 5	0.040 4 ^a	0.003 7 ^b	0.003 7 ^b	0.022 7 ^b

^aSignificant at the 0.05 level.

^bSignificant at the 0.01 level. fHRV Composite 1: based on 44 fetal heart rate variability (fHRV) measures, 6 PCA components. fHRV Composite 2: based on 4 fHRV measures, 2 PCA components.