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On the distinct differences in autonomic regulation between pregnant and non-pregnant women - a heart rate variability analysis

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Abstract

Objective. Appropriate adaptation of the maternal autonomic nervous system to progressing gestation is essential to a healthy pregnancy. This is partly evidenced by the association between pregnancy complications and autonomic dysfunction. Therefore, assessing maternal heart rate variability (HRV) —a proxy measure for autonomic activity—may offer insights into maternal health, potentially enabling the early detection of complications. However, identifying abnormal maternal HRV requires a thorough understanding of normal maternal HRV. While HRV in women of childbearing age has been extensively investigated, less is known concerning HRV during pregnancy. Subsequently, we investigate the differences in HRV between healthy pregnant women and their non-pregnant counterparts. *Approach.* We use a comprehensive suite of HRV features (assessing sympathetic and parasympathetic activity, heart rate (HR) complexity, HR fragmentation, and autonomic responsiveness) to quantify HRV in large groups of healthy pregnant ($n = 258$) and non-pregnant women ($n = 252$). We compare the statistical significance and effect size of the potential differences between the groups. *Main results.* We find significantly increased sympathetic and decreased parasympathetic activity during healthy pregnancy, along with significantly attenuated autonomic responsiveness, which we hypothesize serves as a protective mechanism against sympathetic overactivity. HRV differences between these groups typically had a large effect size (Cohen's $d > 0.8$), with the largest effect accompanying the significantly reduced HR complexity and altered sympathovagal balance observed in pregnancy (Cohen's $d > 1.2$). *Significance.* Healthy pregnant women are autonomically distinct from their non-pregnant counterparts. Subsequently, assumptions based on HRV research in non-pregnant women cannot be readily translated to pregnant women.

Introduction

The autonomic nervous system (ANS) regulates involuntary physiological processes in the human body and therefore plays a crucial role in maintaining and modulating heart rate (HR), blood pressure (BP), and respiration (Shaffer and Ginsberg 2017). During pregnancy, all these involuntary processes need to adapt to the continuously evolving demands of the maternal-fetal pair, necessitating changes in maternal autonomic regulation (Fu 2018). Insufficient adaptation of the maternal ANS to pregnancy is associated with pregnancy complications, such as hypertensive disorders of pregnancy and gestational diabetes, which affect over 10% of pregnancies (Moors *et al* 2020, Reyes *et al* 2020). Consequently, assessing maternal autonomic activity during pregnancy may offer insights into gestational health which are otherwise subclinical (Rang *et al* 2002, Pal *et al* 2009). However, to enable the identification of abnormal maternal autonomic regulation, an in-depth understanding is first needed of the normal activity of the ANS during a healthy pregnancy.

Our current understanding of healthy maternal autonomic regulation is based on conclusions drawn from studies using a variety of methods. Researchers who tested maternal cardiovascular reflexes concluded that activity from the parasympathetic branch of the ANS is reduced (Ekholm *et al* 1994). Concerning the sympathetic branch, results from studies that directly measured electrical activity in sympathetic nerves in the skeletal muscles indicated an increased sympathetic state (Reyes *et al* 2018). Additionally, results from assessments of baroreflex sensitivity showed decreased autonomic regulation of BP toward the end of pregnancy (Brooks *et al* 2020).

Still, while these methods offer valuable insights, they require controlled test setups and would be impractical to use as part of standard parental care. A better-suited, unobtrusive method would consist of assessing heart rate variability (HRV) since this can be monitored longitudinally with wearable devices such as ECG-Holter monitors or wrist-worn photoplethysmography (Shaffer and Ginsberg 2017). Given that the ANS is responsible for regulating HR, assessing the variation in HR offers insight into autonomic regulation (Shaffer and Ginsberg 2017). Standard time- and frequency-domain HRV features inform on the interplay of the sympathetic and parasympathetic systems, while more recently developed features describe further aspects of autonomic regulation such as HR complexity, HR responsiveness, and HR fragmentation (Bauer *et al* 2006a, Shaffer and Ginsberg 2017, Costa *et al* 2017a). HRV assessment is already used in the early detection of sepsis, assessment of fetal health, and risk stratification of cardiac disease (Rajendra Acharya *et al* 2006, Ahmad *et al* 2009, Eick *et al* 2015, Ponsiglione *et al* 2021), to name but a few. Similarly, assessing when maternal HRV (mHRV) deviates from the expected norm during pregnancy may aid in the stratification of high-risk pregnancies.

However, while HRV in healthy women has been extensively studied (Koenig and Thayer 2016), less is known about how pregnancy affects HRV. Additionally, published studies are limited both in sample size (typically $n < 30$ per group, with the largest study still involving less than 100 participants per group (Carpenter *et al* 2015)) as well as in the type of HRV features investigated (Ekholm *et al* 1997, Speranza *et al* 1998, Voss *et al* 2000, Balajewicz-Nowak *et al* 2016, Kuo 2000). Results from these studies—typically using only standard time and frequency domain HRV features—are at times conflicting and often fail to demonstrate clear findings (Sharifiheris *et al* 2022), likely in part due to small sample sizes. A recent review on the potential of mHRV for assessing maternal health confirmed that an understanding of what constitutes healthy mHRV remains lacking (Sharifiheris *et al* 2022). Furthermore, these researchers advocate for mHRV investigations using HRV features outside of the standard time and frequency domain features, since features such as those capturing HR complexity may be better suited to reflecting the intricate physiological changes which occur during pregnancy (Sharifiheris *et al* 2022).

Subsequently, to understand the potential of mHRV in detecting deteriorations in maternal health, a definitive understanding is needed of how mHRV changes during a healthy pregnancy. To this end, we employ a comprehensive set of HRV analyses to quantify the potential differences in autonomic regulation between healthy, non-pregnant women and healthy women at mid-pregnancy ($n > 250$ per group). By analyzing the largest dataset reported thus far in the literature, we aim to clarify how healthy pregnancy impacts standard time and frequency domain features. Furthermore, we investigate HRV features that capture HR complexity, HR responsiveness, and HR fragmentation, some of which are being compared between pregnant and non-pregnant women for the first time. Additionally, we determine the effect size of the differences in HRV features between these two groups to understand the magnitude of the impact of pregnancy on HRV as well as which features are most altered during gestation. Finally, we discuss our results in the context of findings on maternal autonomic regulation based on alternative methods of autonomic assessment. The work outlined in this paper represents the most comprehensive assessment of mHRV in healthy pregnancies to date and forms the basis for the potential use of mHRV in assessing maternal health.

Methods

Datasets

We retrospectively analyzed two datasets. The pregnant group is comprised of abdominal ECG measurements (NEMO Healthcare BV, the Netherlands) of approximately 30 min collected from 492 women with singleton pregnancies between 18 and 24 weeks of gestation (Verdurmen *et al* 2016). Recordings (500 Hz) were taken while women were lying in a semi-recumbent position. The institutional review board at the Máxima Medical Center, Veldhoven, the Netherlands, approved the original study (NL48535.015.14) and all participants provided written informed consent. A waiver was granted for this secondary analysis by the same review board per the Dutch law on medical research with humans (reference number N21.008). The study protocol for the original study, which ran from 2014 to 2017, is described elsewhere (Verdurmen *et al* 2016).

Table 1. Characteristics of the datasets. Data on age, BMI, and measurement length are presented as mean and standard deviation.

Characteristic	Pregnant group	Non-pregnant group
Number of included participants	258	252
Age	30.8 (4.1) years	24.6 (4.8) years
BMI (before pregnancy)	23.9 (4.3) kg m^{-2}	21.9 (2.3) kg m^{-2}
GA at measurement	20 weeks 4 d (9 d)	
Nulliparous	53.1%	
Fetal CHD	68 cases (26.4%)	
Measurement length	29.9 (5.0) min	22.4 (4.2) min

Women with a body mass index (BMI) over 30 kg m^{-2} were excluded ($n = 67$), as well as those who were recorded outside of the gestational age of 18–24 weeks of pregnancy ($n = 53$), as specified in the original protocol (Verdurmen *et al* 2016). Furthermore, maternal HRV is known to vary across pregnancy (Balajewicz-Nowak *et al* 2016, Garg *et al* 2020, Bester *et al* 2022), hence the gestational age is limited to within this range. Thereafter, those with pre-existing health conditions such as diabetes, maternal pregnancy complications such as hypertensive disorders of pregnancy, or those who were taking medications other than vitamins ($n = 106$), were also excluded from our analysis. A further two women are excluded owing to known atrial fibrillation. Furthermore, eight were excluded during data preprocessing (see next section). In total, we included 252 participants. Of these, 68 had fetuses with fetal congenital heart disease (CHD). However, it has been demonstrated that fetal CHD does not affect mHRV Bester *et al* (2022) and, therefore, they are not excluded here. Patient characteristics are presented in table 1. For a few patients information on age ($n = 17$) or BMI ($n = 5$) is missing; these women are assigned the mean age and BMI.

The non-pregnant control group consists of participants from the Autonomic Aging dataset which is openly available from Physionet (Goldberger *et al* 2000, Schumann and Bär 2022). ECG data were collected from 1121 participants in a resting, supine position. Participants were screened for any medical condition, use of illegal drugs or any medications potentially influencing cardiovascular function. All participants were at least 18 years old. Recordings were done at a sampling frequency of 1000 Hz using either a MP150 (ECG100C, BIOPAC systems inc., Golata, CA, USA) or a Task Force Monitor system (CNSystems Medizintechnik GmbH, Graz, AUT). These recordings varied considerably in length; subsequently, recordings of lengths between 20 and 40 min were included ($n = 468$). We excluded all men ($n = 165$) and women 45 years old or older ($n = 27$). Furthermore, we excluded women with a BMI over 30 kg m^{-2} ($n = 15$). Ten women were excluded during data preprocessing (see next section), finally resulting in the inclusion of 252 non-pregnant women. Participant characteristics are outlined in table 1. The ages of the non-pregnant group are only available as grouped data, e.g. participant 1 is between 20 and 24 years old, participant 2 is between 40 and 44 years old, etc. For seven participants, no age data was available. While precise values are not available, we can estimate the mean and standard deviation of such grouped data. Subsequently, all data in table 1 are reported as mean and standard deviation, where applicable.
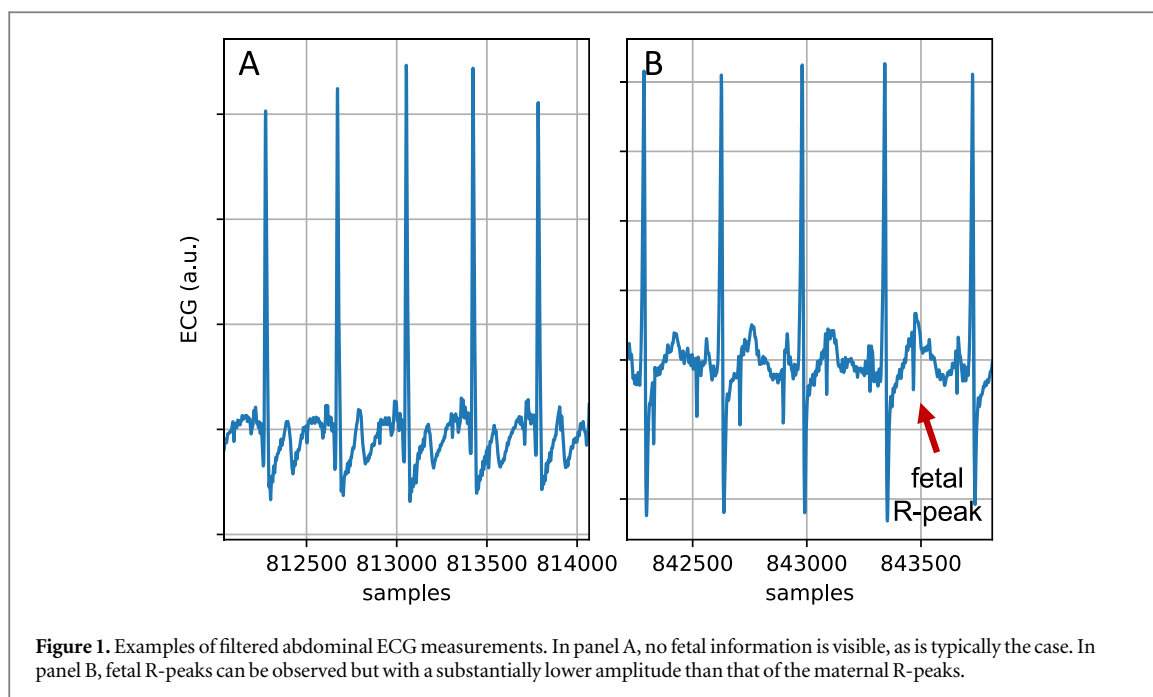
Preprocessing

While abdominal ECG measurements are typically acquired to obtain fetal ECG information, the amplitude of the maternal ECG signal far exceeds that of the fetal ECG. In fact, extracting fetal information from abdominal ECG measurements is a persistent challenge (Jaros *et al* 2018, Fotiadou *et al* 2021). While preprocessing of these abdominal ECG measurements is done to improve the quality of the measurement, as detailed below, it is important to note that the fetal information does not pose an obstacle in detecting maternal R-peaks, as can be seen in figure 1. Figure 1(A) is a representation of a typical abdominal ECG measurement; the fetal information is not visible. Figure 1(B) is a rarer example, where fetal peaks are visible. Still, the amplitude of the maternal R-peak dwarfs that of the fetal peak.

The multichannel abdominal ECG measurements from the pregnant group are filtered by applying a 4th order Butterworth bandpass filter of 1–70 Hz to suppress out-of-band noise and artifacts. Next, a notch filter is applied at 50 Hz to suppress powerline interference and a fixed linear combination of the various abdominal channels is applied to enhance maternal QRS peaks (Rooijakkers *et al* 2014).

The processing of maternal *RR* intervals from fetal ECG measurements was done in MATLAB (MathWorks, USA). All further processing, analyses, and generating of figures were done in Python (PSF, USA).

For both datasets, a previously published peak detector is used to detect the R-peaks (Rooijakkers *et al* 2012, Bester *et al* 2022) and generate the corresponding tachograms. *RR*-intervals that are physiologically improbable (shorter than 0.4 s or longer than 2 s) or that differ from the preceding interval by more than 20% are rejected (Campana *et al* 2010, Peters *et al* 2008, 2011). Furthermore, missing *RR*-values are interpolated using cubic

Figure 1. Examples of filtered abdominal ECG measurements. In panel A, no fetal information is visible, as is typically the case. In panel B, fetal R-peaks can be observed but with a substantially lower amplitude than that of the maternal R-peaks.

spline in cases where the HRV features require a continual time series (specifically, frequency domain and complexity HRV features). Since interpolation is known to influence HRV results, signals which required more than 1% interpolation across the entire recording are excluded when calculating these HRV features. This results in a comparison between 163 non-pregnant and 182 pregnant participants. For the remaining HRV features, all signals for which less than 15% of *RR*-intervals needed to be removed are included in the analysis. Subsequently, data from 258 pregnant and 252 non-pregnant women are used.

HRV features

Standard time- and frequency-domain features
The mean HR is calculated in beats per minute along with the standard deviation of the *RR*-intervals (SDNN) to represent overall variability. The root mean square of the successive differences of the *RR*-intervals (RMSSD) and the percentage of consecutive *RR*-intervals that differ by more than 50 ms (pNN50) are calculated as a measure of parasympathetic activity since such short-term variations are mediated by the vagus nerve. To study the spectral activity linked to the parasympathetic system, the power in the high frequency (HF) band of 0.15–0.40 Hz is calculated. Furthermore, the power in the low frequency (LF) band of 0.04–0.15 Hz (influenced by both branches of the ANS), as well as the LF/HF ratio, are calculated (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology 1996, Shaffer and Ginsberg 2017).

For calculating these spectral features, Welch's method is used. Recordings are divided into five-minute segments with 50% overlap; the features are calculated for each five-minute segment and subsequently, the mean of all segments is presented as the final feature value for each recording. For the time-domain features as well as all the following HRV features, the feature is calculated across the entire recording.

Nonlinear and complexity features
We use a Poincaré plot—a popular geometrical method to evaluate HRV dynamics—in which each *RR*-interval is plotted against its predecessor to form a scatter plot that is fitted with an elliptical shape. From this ellipse, three parameters are calculated: the short- and long-term *RR* variability (SD1 and SD2), as well as the ratio between them (SD1/SD2) (Khandoker *et al* 2013). Furthermore, we assess complexity in the tachograms with two features: sample entropy (SampEn) and detrended fluctuation analysis (DFA) (Peng *et al* 1995, Richman and Moorman 2000). SampEn quantifies the conditional probability that two epochs which are similar within a tolerance *r* for a window length *m* will remain similar when including the next data point (i.e. the next *RR* interval) (Richman and Moorman 2000, Bakhchina *et al* 2018). The parameters *m* and *r* are set to 2 and 0.2 times the standard deviation of the *RR*-intervals (Richman and Moorman 2000). Lower SampEn indicates a more regular and predictable time series (Shaffer and Ginsberg 2017). Additionally, DFA is used to quantify the fractal scaling properties of the time series to give an estimation of its long-range correlations. We calculate the short-term fractal scaling exponent α_1, which represents the correlation over 4–16 heartbeats (Peng *et al* 1995). A result of $\alpha = 0.5$ and $\alpha = 1.5$ represent no correlation (i.e. white noise) or a random walk process (i.e. Brownian

noise), respectively. Positive correlations exist when $0.5 < \alpha < 1.5$, with $\alpha \approx 1$ suggesting a high level of complexity. Values above 1 suggest that the system becomes increasingly regular (Peng *et al* 1995, Yeh *et al* 2009).

Heart rate fragmentation

Overall, the presence of variability in the tachogram suggests healthy autonomic control. However, situations in which there is a breakdown in the controlled physiological variation of the HR (such as aging) may also result in higher levels of short-term variability (Costa *et al* 2017a). Heart rate fragmentation (HRF) features capture this jagged type of variability which is likely a result of inadequate autonomic control, but rather of a breakdown in the neuroautonomic-electrophysiological control systems that regulate HR (Costa *et al* 2017a).

Four indices were developed by Costa *et al* to capture this fragmentation in the HR (Costa *et al* 2017a): percentage inflection points (PIP), inverse of accelerating or decelerating long segments (IALS); percentage short segments (PSS); and percentage alternating segments (PAS). PIP captures how often the acceleration sign of the HR is changing. IALS represents the inverse of the average length of sustained accelerating or decelerating *RR*-intervals. PSS is the complement of the percentage of *RR*-intervals with a sustained acceleration or deceleration in HR for at least three intervals. Finally, PAS is the percentage of the *RR*-intervals which are continuously alternating between accelerations and decelerations (starting from a minimum of four intervals). Note that increases in these indices reflect increased HR fragmentation.

Phase rectified signal averaging

Phase rectified signal averaging (PRSA) is a method that quantifies how the tachogram responds to accelerations and deceleration in the HR as a proxy measure for autonomic responsiveness. We briefly describe the method here; for a more detailed description and visualization of this technique, please refer to the original publication (Bauer *et al* 2006a). This method allows us to capture the quasi-periodicities in the tachogram, which can often be obscured by noise and non-stationarities. This is done by identifying a phase of interest, placing anchor points (APs) everywhere this phase occurs, isolating a signal segment of length $2L$ around each AP, aligning segments by their phase, and finally averaging these segments. We specify two sets of APs, namely each HR deceleration and HR acceleration. Furthermore, we define L as 50 *RR* values, as is also done in the literature (Joshi *et al* 2018).

The resulting PRSA waveform visualizes the behavior of HR in response to accelerations and decelerations. The magnitude and speed of the response observed in the waveform give an estimate of the robustness of the autonomic response (Bauer *et al* 2006a). (Note that the PRSA waveform's relationship to the time domain is units of *RR* values (specified here as RR_i) and not in seconds.) Features are calculated to quantify the PRSA waveform (X). The most established feature, deceleration capacity (DC), is calculated as follows:

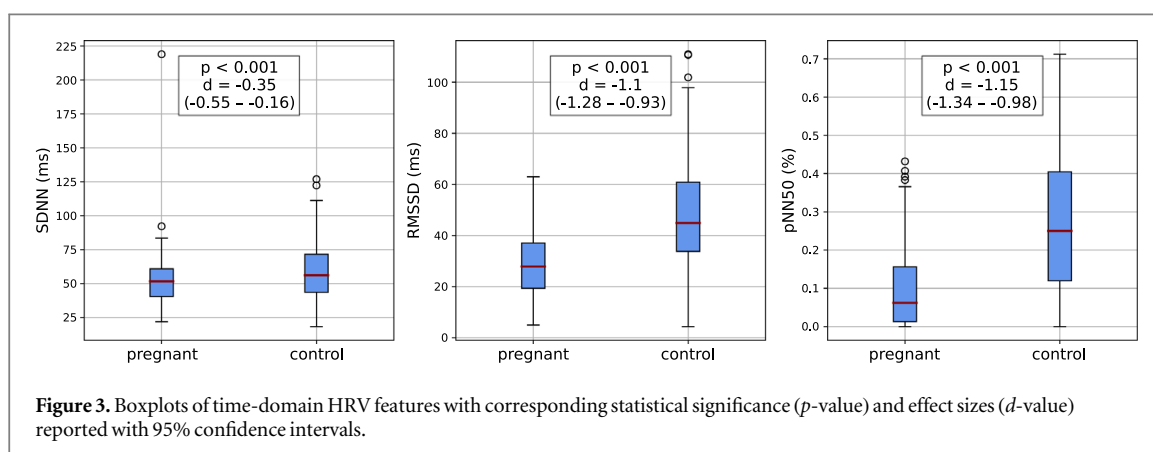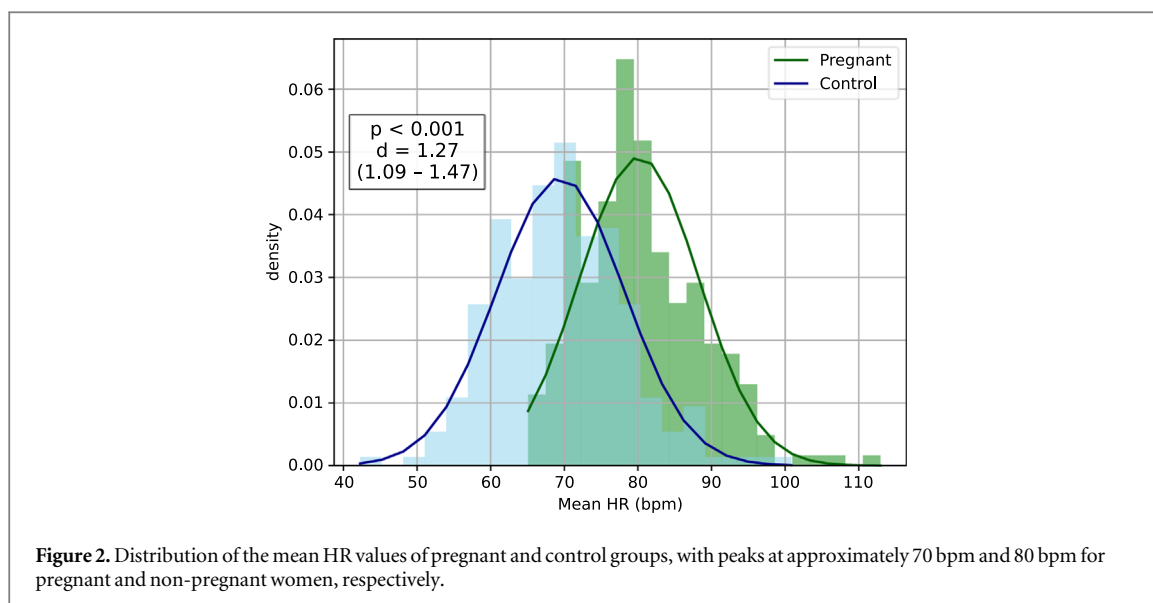$$DC = [X(0) + X(1) - X(-1) - X(-2)]/4, \tag{1}$$

with $X(0)$ representing the AP, $X(1)$ is the value following the AP, while $X(-1)$ and $X(-2)$ precede the AP (Bauer *et al* 2006a). The acceleration capacity (AC) is similarly calculated. Additionally, the difference between the maximum and minimum RR_i within the neighborhood of five RR_i preceding the AP and five after, including the AP, is calculated to determine the immediate deceleration response (IDR) and immediate acceleration response (IAR). The rates corresponding to these responses are also calculated with the slope of the deceleration and acceleration responses (SDR and SAR) (Joshi *et al* 2018).

Statistical analysis and data representation

The normality of data was tested with D'Agostino's K^2 test. Only mean HR was normally distributed for both groups; subsequently, a Student t-test was used to test for significance ($p < 0.05$) of the difference in HR, while a non-parametric test (the Mann-Whitney U test) was performed for all other features. Corresponding effect sizes were calculated with Cohen's d, where 0.2 amounts to a small effect, 0.5 to a medium effect, and 0.8 to a large effect. However, since Cohen's d assumes a normal distribution for the data, we perform a bootstrapping procedure (10 000 iterations) and report the subsequent mean d-value along with the 95% confidence intervals (CI), as is appropriate in non-parametric analyses (Kelley 2005). Note that d-values may also be negative and that the magnitude of the change is inferred from the absolute d-value. To further contextualize the effect sizes of the differences between our two groups, we additionally calculated to effect sizes of the differences in HRV between women (i.e. our non-pregnant control group) and men. These two groups are known to have differences in their autonomic regulation (Koenig and Thayer 2016). The details and results of this analysis can be found in the appendix.

Results

We graphically present our results along with the appropriate statistics. For the mean HR (the only feature with a normal distribution), we plot the distribution of each group; all other features are presented as boxplots. Figure 2

Figure 2. Distribution of the mean HR values of pregnant and control groups, with peaks at approximately 70 bpm and 80 bpm for pregnant and non-pregnant women, respectively.

Figure 3. Boxplots of time-domain HRV features with corresponding statistical significance (*p*-value) and effect sizes (*d*-value) reported with 95% confidence intervals.
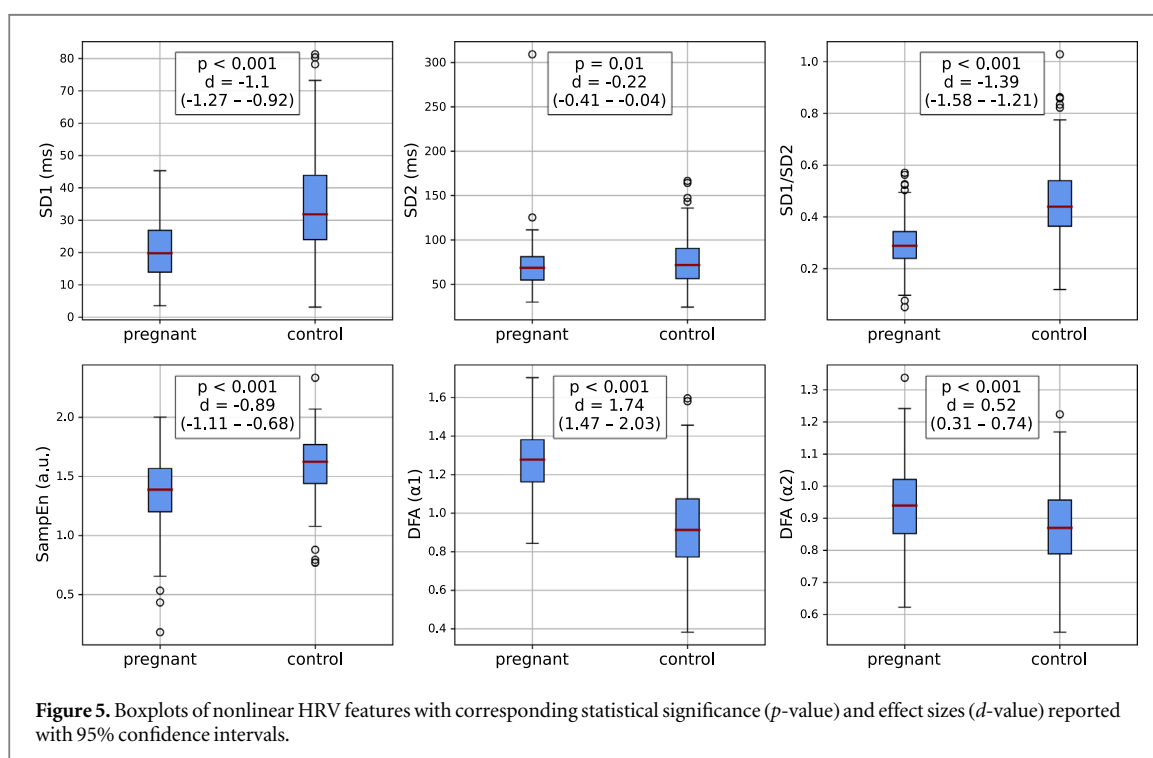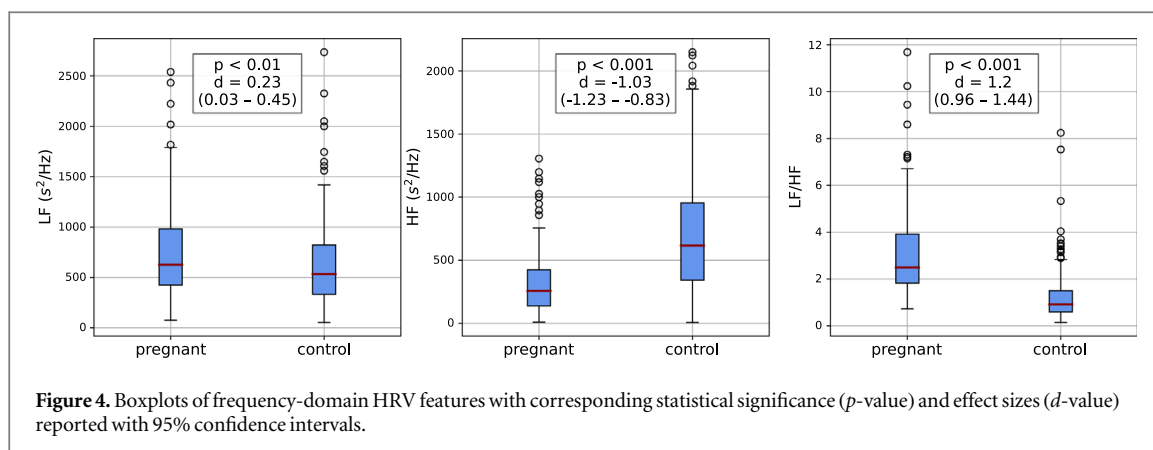
shows the distribution of the mean HR for each group, clearly demonstrating a significantly increased HR in pregnant women ($d = 1.27$ (1.09–1.47)). Additionally, features of HRV (figure 3) that are linked to short-term variation (RMSSD and pNN50) are significantly reduced ($d = -1.1$ (-1.28 - -0.93) and -1.15(-1.34 - -0.98), respectively). SDNN shows a statistically significant yet small change between groups ($d = -0.35$ (-0.55 - -0.16)).

In the frequency domain (figure 4) we see a similar statistically significant reduction in HF, the feature linked to vagal activity ($d = -1.03$ (-1.23 to -0.83)). Low frequency (LF) is significantly elevated, while LF/HF increases significantly with a large effect size ($d = 1.2$ (0.96–1.44)).

Most nonlinear features (figure 5) show large changes. SD1/SD2 is significantly decreased ($d = -1.39$ (-1.58 to -1.21) during pregnancy, which is driven by a large change in SD1 ($d = -1.1$ (-1.27 to -0.93)). The latter is also linked to vagal activity. DFA (α_1) is increased in pregnancy with a remarkably large effect size ($d = 1.74$ (1.47–2.03)), a change that signals a decrease in the complexity of the HR. Additionally, the statistically significant and large decrease in SampEn ($d = -0.89$ (-1.11 to -0.68)) suggests the same.

One of the HRF features in figure 6 (IALS and PSS) similarly has a large effect size between the two groups ($d = -0.87$ (-1.07 to -0.67)). This feature represents the absence of sustained HR accelerations and decelerations and is significantly decreased in pregnancy. Furthermore, PIP and IALS are also significantly decreased during pregnancy with small effect sizes, while PAS is significantly increased, also with a small effect size.

For the PRSA analysis, the average PRSA waveform for each group is plotted (figure 7) in addition to the boxplots representing the feature values (figure 8). From figure 7, we can see that the autonomic response of pregnant women is attenuated when compared to non-pregnant controls. This can be seen by noting the smaller amplitude of the blue waveform. This is further confirmed by the statistically significant decreases in features capturing the PRSA response for pregnant women in figure 8, overall, with medium to large effect sizes.

Figure 4. Boxplots of frequency-domain HRV features with corresponding statistical significance (*p*-value) and effect sizes (*d*-value) reported with 95% confidence intervals.

Figure 5. Boxplots of nonlinear HRV features with corresponding statistical significance (*p*-value) and effect sizes (*d*-value) reported with 95% confidence intervals.

Furthermore, a smoother response is observed in the PRSA waveform of pregnant women (figure 7). This prompted a visualization of the frequency domain of these waveforms using power spectral density (PSD). From the PSDs, we can approximately observe the spectral activity in the areas associated with the traditional LF and HF areas of HRV. Increased activity in the LF region and decreased activity in the HF region is observed for pregnant women, again suggesting increased sympathetic and decreased parasympathetic (or vagal) activity.

Finally, figure 9 presents the effect sizes with 95% CI for all features in descending absolute magnitude. Most features show changes between pregnant and non-pregnant women with large effect sizes ($d > 0.8$). DFA (α_1)—linked to HR complexity—has the largest effect size. SD1/SD2 and LF/HF also have similarly large effect sizes; both these features relate to the balance between the sympathetic and parasympathetic systems. All the features closely linked to vagal activity (pNN50, SD1, RMSSD, and HF) show similar effect sizes around $d = 1.1$.

In the appendix, a similar graph (figure A.1) can be found which presents the effect sizes of the differences in HRV between women (i.e. the non-pregnant control group) and men. When comparing figures 9 to A.1, it appears that there are larger changes in autonomic regulation between non-pregnant women and pregnant women than there are between non-pregnant women and men.

Discussion

Dramatic changes occur in maternal physiology during pregnancy. Not only are there substantial adaptations in most organ systems, but large shifts also occur in autonomic regulation. In this paper, we outline the differences
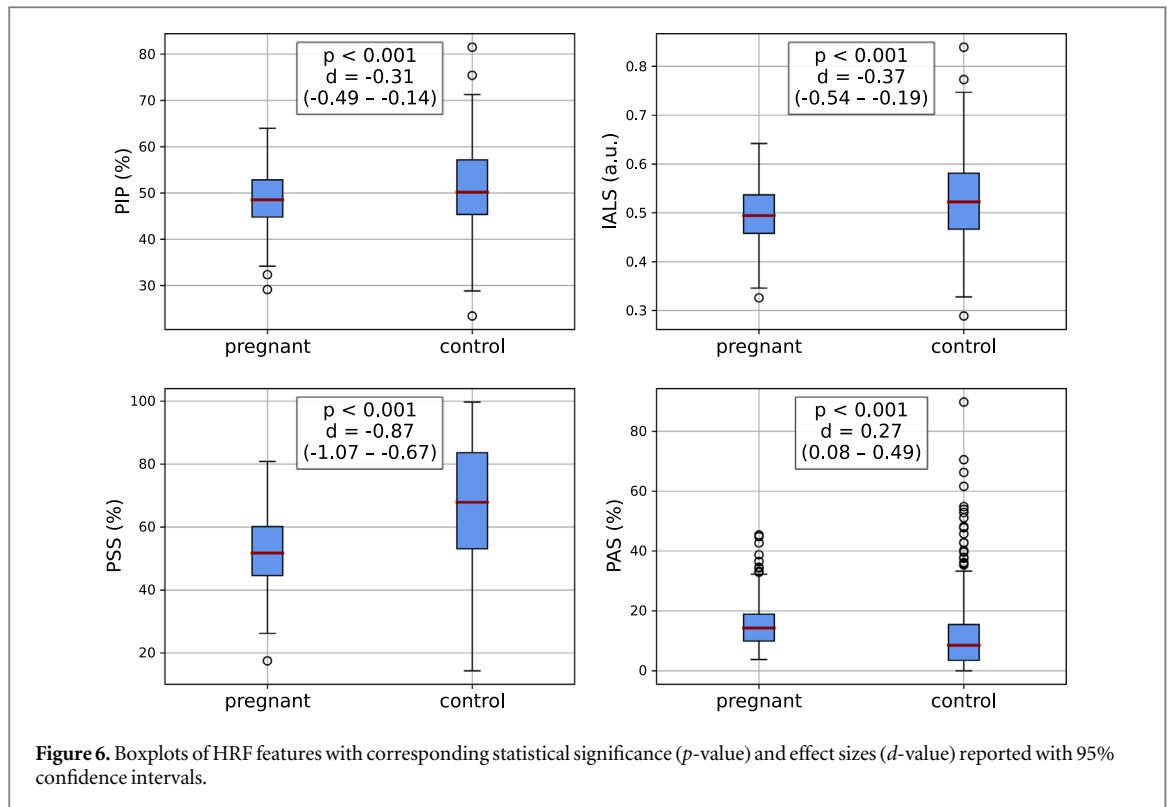
Figure 6. Boxplots of HRF features with corresponding statistical significance (*p*-value) and effect sizes (*d*-value) reported with 95% confidence intervals.
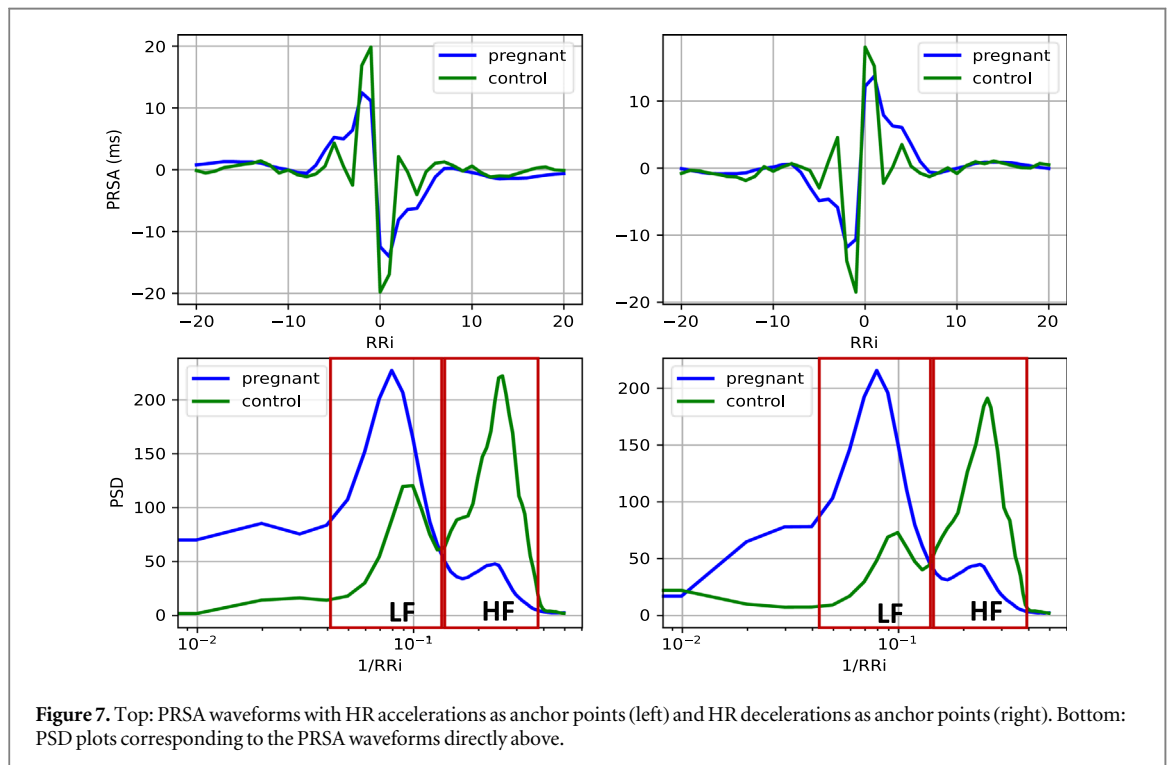

Figure 7. Top: PRSA waveforms with HR accelerations as anchor points (left) and HR decelerations as anchor points (right). Bottom: PSD plots corresponding to the PRSA waveforms directly above.

in autonomic regulation as assessed with a comprehensive set of HRV between pregnant and non-pregnant women in large cohorts. We compare features such as SampEn and those related to HRF for the first time between pregnant and non-pregnant women, finding that lower HR complexity and HRF are present during pregnancy. Furthermore, we demonstrate that pregnant women have significantly reduced autonomic responsiveness, building on preliminary work by our group (based on only nine participants per group) which indicated that only some PRSA features were affected by pregnancy (Bester *et al* 2022). Additionally, based on the large groups assessed in this work, we find that mHRV in pregnancy reflects reduced parasympathetic and increased sympathetic activity, resolving the often conflicting findings of smaller studies (Sharifiheris *et al* 2022).

Figure 8. Boxplots of PRSA features with corresponding statistical significance (*p*-value) and effect sizes (*d*-value) reported with 95% confidence intervals.
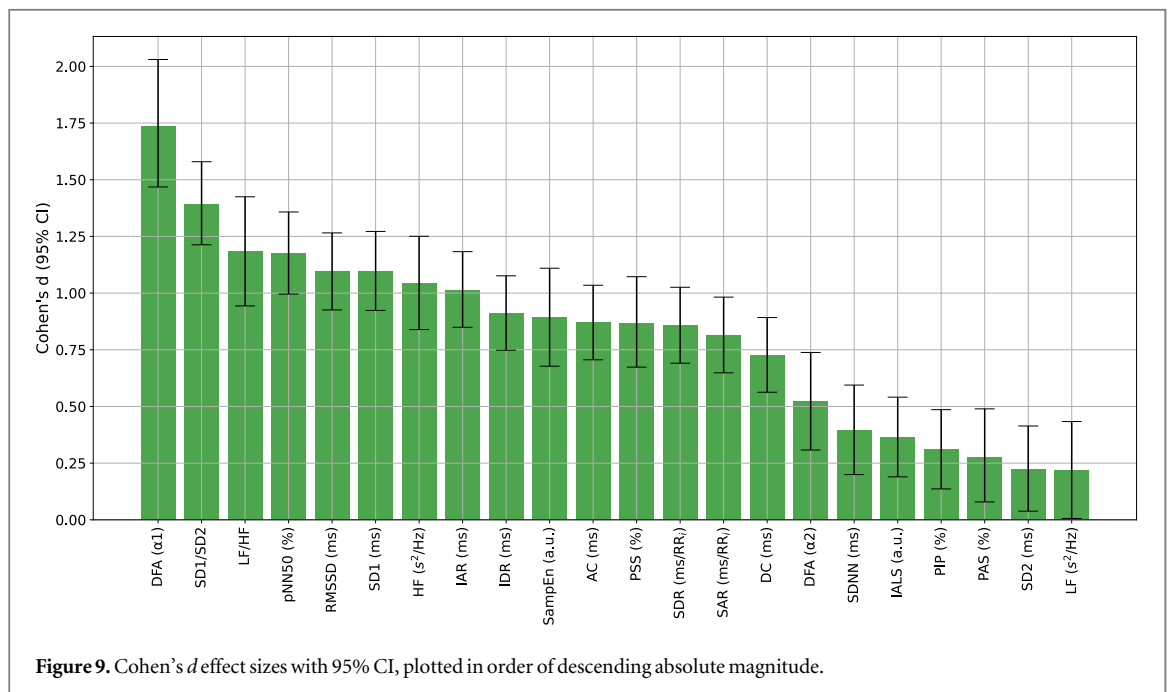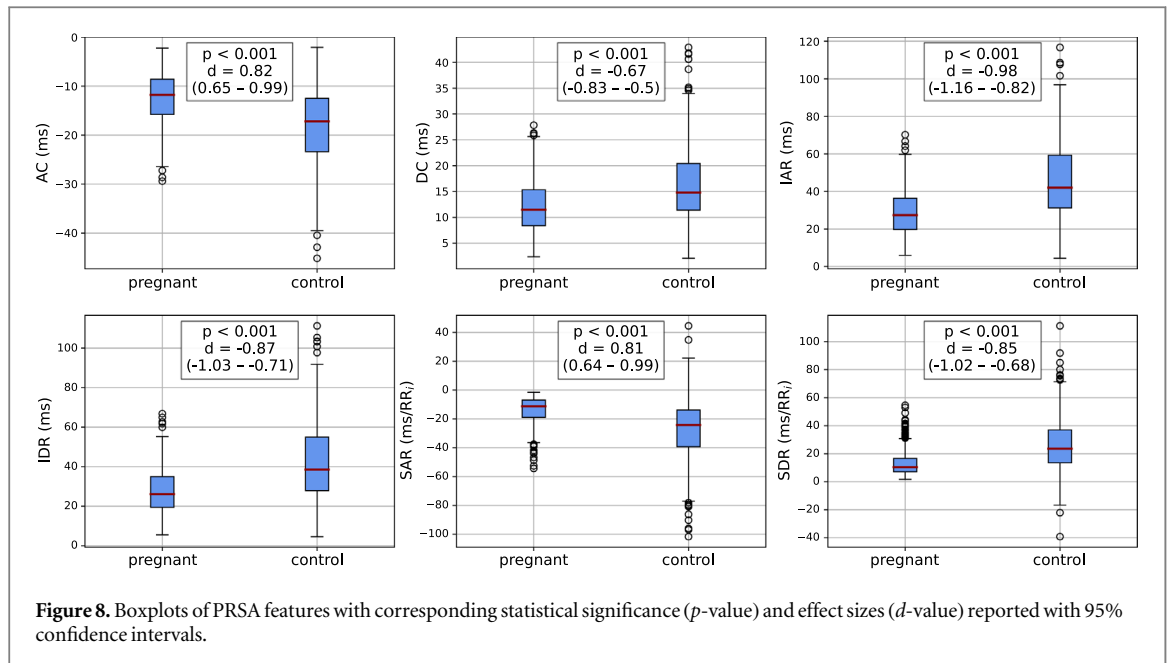

Figure 9. Cohen's *d* effect sizes with 95% CI, plotted in order of descending absolute magnitude.

Moreover, we investigated the effect sizes of differences between these groups; overall, we find that healthy women at mid-pregnancy are autonomically distinct from their non-pregnant counterparts.

We find that HR complexity is remarkably reduced during pregnancy; the significantly lower SampEn in the pregnant group suggests a large drop in complexity at mid-pregnancy (figure 5, d = −0.89 (−1.11 to −0.68)). Furthermore, the feature α_1 from DFA, which captures short-term changes in HR over multiple timescales, shows a large, significantly increased in the pregnant group as compared to the non-pregnant group (d = 1.74 (1.47–2.03)), which signals reduced self-similarity in the HR signal. The latter result confirms that of a smaller study, which found significantly elevated α_1 in late pregnancy compared to non-pregnant controls (n = 16) (Yeh *et al* 2009). HR complexity and self-similarity have rarely been explored in pregnancy and, as such, there is no known physiological explanation for this change.

However, recent studies have shown that α_1 is well-suited for capturing the fatigue of ultramarathon runners (Gronwald *et al* 2021, Rogers *et al* 2021), even in cases where HR remains steady (Rogers *et al* 2021) or when standard features such as SDNN and RMSSD show little relation to fatigue (Gronwald *et al* 2021). The researchers who performed this work suggest that during a fatigued state, the integration between the

physiological subsystems of the human body over different timescales starts to break down, manifesting as the decoupling between systems (e.g. the cardiac and respiratory systems). This may act as a protective mechanism, ensuring that interactions between systems fail before whole systems do (Rogers *et al* 2021). We hypothesize a similar mechanism to be in place during pregnancy. The increased physiological stress of pregnancy, along with the added burden of the placental-fetal unit on the maternal cardiovascular system, likely results in systems functioning more independently, leading to a decrease in HR complexity. These results support previously published work, which found that these nonlinear features are more sensitive to GA than standard HRV features when tracked from 15 to 41 weeks of gestation (Bester *et al* 2022). Furthermore, these researchers found that SampEn has a statistically significant relationship with GA even across the narrow range of 18–24 weeks of gestation, while SDNN and RMSSD showed no relationship (Bester *et al* 2022).

Additionally, we investigated the effect of pregnancy on HRF for the first time. Three HRF features are significantly reduced in pregnant women (figure 6), with PSS showing a large change ($d = -0.87 (-1.07$ to $-0.67)$). This finding is somewhat surprising as it suggests that pregnancy reduces HR fragmentation. Alternatively, an increase in HR fragmentation would suggest a breakdown in the hierarchy of the physiological systems regulating HR, as is the case in older populations and those with coronary artery disease (Costa *et al* 2017a, 2017b). Since participants in the pregnant group are healthy, we would not expect increased fragmentation. However, it is quite remarkable that HR fragmentation seems to reduce. We should note here that HRF is not yet as well established as the other HRV features assessed in this study and that the basic mechanisms underlying fragmentation still need to be fully explored (Costa *et al* 2018). However, a large decrease in PSS in pregnant women suggests an increase in sustained accelerations and decelerations of the heart rhythm (or conversely, a decrease in RR-intervals quickly alternating between acceleration and deceleration).

This may be at least partially ascribed to a state of decreased vagal activity, which regulates beat-to-beat HR variation, in conjunction with the increased sympathetic activity, which is responsible for changing the HR over longer time scales. The mHRV study with the largest sample size in the literature (99 pregnant women and 63 controls) found this autonomic state to be present in the first trimester (Carpenter *et al* 2015), however, other researchers found increased vagal activity (Alam *et al* 2018) and decreased sympathetic activity in early pregnancy (Stein *et al* 1999, Alam *et al* 2018). Considering analyses done on women in mid-pregnancy, as is also the case for our study group, Ekholm *et al* found in 1992 that pregnant women have decreased parasympathetic activity and increased sympathetic activity at mid-pregnancy (Ekholm *et al* 1992). These findings are also supported by further investigations (Balajewicz-Nowak *et al* 2016, Garg *et al* 2020). However, other studies have found sympathetic activity, as assessed with LF, to be decreased (Ekholm *et al* 1997, Voss *et al* 2000) or not significantly altered during pregnancy (Eneroth-Grimfors *et al* 1994), rather than increased. However, these studies were performed using small sample sizes ($n < 30$). Furthermore, LF is known to be a sensitive metric that should be interpreted with caution (Heathers 2014). Still, the results of our standard HRV features reaffirm those of (Ekholm *et al* 1992, Balajewicz-Nowak *et al* 2016, Garg *et al* 2020) in that vagal activity (as assessed by RMSSD, pNN50, and HF, figures 3 and 4) is reduced in pregnant women, while sympathetic activity—in so far as we can infer sympathetic activity from changes in LF and LF/HF (figure 4)—is increased. The increased HR (figure 2), which we expect based on the literature (Loerup *et al* 2019, Green *et al* 2020), as well as the decreased SD1/SD2, further suggest increased sympathetic and decreased parasympathetic activity. Furthermore, the overall findings on vagal and sympathetic activity also align with the conclusions drawn from investigations using microneurography (i.e. direct measurement of sympathetic activity in the skeletal muscles) and cardiovascular reflex tests to assess maternal autonomic tone (Rang *et al* 2002, Reyes *et al* 2018).

Results from the PRSA analysis also suggest reduced vagal activity (AC and DC are significantly reduced in pregnancy; figure 8). This is further confirmed by the clear reduction in HF activity observed in the corresponding PSDs in figure 7. Looking at the magnitude and rate of the responses (IAR, IDR, SAR, and SDR), we can further conclude that autonomic responsiveness is diminished in pregnant women. This is another notable result since reduced responsiveness is typically associated with states such as cardiac disease and fetal distress (Bauer *et al* 2006a, Weyrich *et al* 2020). Yet, from visual inspection of the PRSA waveforms, it appears that the dampening seen in a healthy pregnancy is smaller than that seen in cases of cardiac disease (Bauer *et al* 2006b). However, since effect sizes are not reported for the latter, it is not possible to make a definitive comparison. Still, this dampened autonomic responsiveness during healthy gestation is echoed in other areas of research. Investigators have found attenuated baroreflex sensitivity (Brooks *et al* 2020), reduced physiological responsiveness to stimuli such as pain and relaxation tests (DiPietro *et al* 2012), and—interestingly—reduced neurocardiovascular transduction. The latter refers to a state where the amount of sympathetic activity in the body has a lower than expected effect on cardiovascular end-points, such as HR (Reyes *et al* 2018). The only prior work comparing PRSA between pregnant ($n = 9$) and non-pregnant ($n = 9$) women is a preliminary analysis performed by our group (Bester *et al* 2022); here, AC, IAR, SAR, and SDR were significantly reduced in pregnant women, while DC, IDR, ADR, and AAR showed no significant changes, potentially due to the small sample sizes.

Overall, we can infer from our results that healthy pregnancy is indeed a state of reduced vagal activity and overactive sympathetic activity compared to non-pregnant controls. Such an autonomic state is likely necessary to maintain a healthy pregnancy, for example, to ensure proper perfusion of the placenta. However, this altered autonomic regulation is possibly dangerous, as it is similar to that found in cases of cardiac disease. To this end, we hypothesize that the reduced autonomic responsiveness (which is reflected in our PRSA analyses as well as the known reduced neurocardiovascular transduction in pregnancy) is a mechanism by which the mother is protected against her autonomic state. This theory is further reflected in findings from Casati *et al* (2016), who observed increased autonomic responsiveness in women with pregnancy complications (such as hypertensive disorders of pregnancy) when compared to healthy pregnant controls. Subsequently, we believe PRSA analysis may be particularly useful in assessing maternal health via mHRV.

It should be noted that our study is limited in terms of measurement length ($\approx$25 min). Future studies should aim at incorporating 24 h measurements, which may offer additional information on the underlying slower processes influencing HRV. Additionally, as the mean age of the pregnant women is approximately five years greater than that of the non-pregnant women, the results observed in this paper are potentially exaggerated. However, based on reference ranges for HRV in the non-pregnant population as well as prior work from our group on the impact of age on mHRV (Sammito and Böckelmann 2016, Bester *et al* 2022), it is unlikely that the differences observed between the groups are predominantly a result of their age difference. Furthermore, recordings were acquired in different positions for the respective groups. While the supine position is typical for resting HRV assessments in non-pregnant women, a semi-recumbent position is preferred in the case of pregnant women since aortocaval compression can occur in the supine position which is known to affect autonomic regulation (Chen *et al* 1999). While the impact of this difference in positions on the results is not known, both groups are in the preferred position for HRV measurements. Additionally, we could not account for the potential impact of the different stages of the menstrual cycle which the non-pregnant women may be in. However, the impact of these stages on HRV is small compared to the changes observed in this study (Vallejo *et al* 2005, Tenan *et al* 2014).

Furthermore, this work is a secondary analysis of data collected to define normative fetal ECG ranges between 18 and 24 weeks of gestation; as such, only data from mid-pregnancy are analyzed for the pregnant group. Previous work has shown that HRV also changes significantly with progressing pregnancy (Bester *et al* 2022). Therefore, further studies are needed to definitively conclude how mHRV differs between non-pregnant women and those in early- and late pregnancy, respectively. However, the work presented here has several advantages over the current state of the art in the literature, chiefly the variety of HRV features investigated (instead of only the standard time- and frequency-domain features) as well as the large sample groups, which allow us to confidently draw conclusions concerning mHRV at mid-pregnancy.

Finally, to contextualize the magnitude of the changes we observe between pregnant and non-pregnant women, we repeated our analysis to compare the group of non-pregnant women against men (see appendix). We found that the effect sizes of the differences between pregnant and non-pregnant women (figure 9) are overall larger than those of non-pregnant women compared to men (Appendix, figure A.1). While autonomic regulation is known to differ between the sexes (Koenig and Thayer 2016), from our analysis it appears that women are more autonomically different from their pregnant counterparts than they are from men.

Conclusion

Subsequently, we conclude that healthy mid-pregnant and non-pregnant women are two autonomically distinct groups, and findings of HRV in non-pregnant women cannot be translated to pregnant women. Furthermore, our findings on mHRV not only align with results from other areas of autonomic investigation but also provide additional information on the maternal autonomic state. These changes often have large effect sizes, the most remarkable of which are for DFA (α_1), SD1/SD2, and LF/HF, suggesting that these may be particularly useful in assessing maternal health.

Data availability statement

The data for the non-pregnant women are publicly available from Physionet (https://doi.org/10.1038/s41597-022-01202-y). The data for the pregnant women cannot be made publicly available upon publication because they contain sensitive personal information. The data that support the findings of this study are available upon reasonable request from the authors.

Conflict of interest

R V is a co-founder and shareholder in Nemo Healthcare BV, the company of which devices were used to collect data. R J is employed by Philips Research. The other authors have no competing interests to declare.
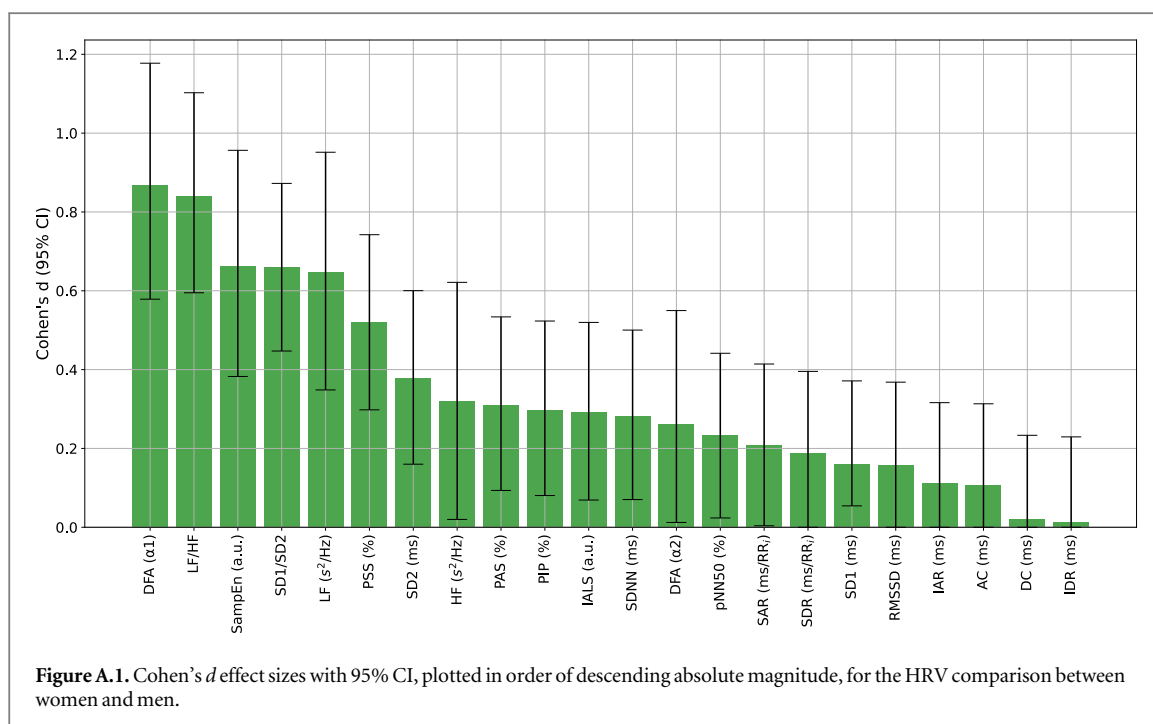
Ethical approval

All human studies have been approved by the appropriate ethics committee and therefore been performed per the ethical standards laid down in the 1964 declaration of Helsinki and its later amendments.

Ethical statement

The institutional review board at the Máxima Medical Center, Veldhoven, the Netherlands, approved the original study (reference number NL48535.015.14) and all participants provided written informed consent. The same board granted a waiver for this secondary analysis in 2021 (reference number N21.008). All human and studies have been approved by the appropriate ethics committee and therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki and its later amendments.

Appendix

We repeated the analysis detailed in this paper to compare HRV between women and men. The women in this comparison are the same as in the non-pregnant control group. Therefore, 252 women were included when comparing HRV features that do not require interpolation of the *RR*-intervals, and 166 women were included in comparisons that necessitate interpolation. These women were included from the Autonomic Aging dataset (available at Physionet) which also contained ECG recordings of men. Subsequently, we also obtained our male group from this Autonomic Aging dataset by applying similar inclusion and exclusion criteria. Subsequently, 131 men were included in the analyses which did not require interpolation, and 78 were included in the analyses which did. Figure A.1 represents the effect sizes of the differences between each feature listed on the *x*-axis. Effect sizes were calculated with Cohen's *d*, where 0.2 amounts to a small effect, 0.5 to a medium effect, and 0.8 to a large effect.

Figure A.1. Cohen's *d* effect sizes with 95% CI, plotted in order of descending absolute magnitude, for the HRV comparison between women and men.

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