

Clinical Research Article

# Pregnancy-specific Reference Intervals for BNP and NT-pro BNP—Changes in Natriuretic Peptides Related to Pregnancy

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**Abbreviations:** BMI, body mass index; BNP, B-type natriuretic peptide; NT-pro BNP, N-terminal-pro B-type natriuretic peptide; RI, reference interval.

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## Abstract

**Context:** Cardiac disease is the leading cause of maternal mortality in the UK, so accurate cardiovascular diagnoses in pregnancy are essential. BNP (B-type natriuretic peptide) and NT-pro BNP (N-terminal-pro BNP) are useful clinical tools for investigating suspected peripartum cardiomyopathy but, as the pregnancy-specific reference intervals are undefined, it is uncertain how they should be interpreted in pregnant women.

**Objectives:** To define trimester-specific 95% reference intervals for BNP and NT-pro BNP in pregnancy.

**Methods:** Longitudinal study of 260 healthy pregnant women, with sampling in each trimester.

**Results:** The upper reference limit for NT-pro BNP was 200 pg/mL in the first and second trimesters, and 150 pg/mL in the third. Levels were significantly reduced in overweight women in the third trimester ( $P = .0001$ ), which supports the partitioning of reference intervals by body mass index (BMI). The upper limit for BNP was 50 pg/mL, with no detectable trimester-related differences. Although other biomarkers (hemoglobin and platelets) fell throughout pregnancy, both natriuretic peptides were initially elevated before falling by the third trimester, suggesting that the observed changes in natriuretic peptides are driven by dynamic interplay between cardiac strain and progressive hemodilution. NT-pro BNP in the first trimester was inversely associated with neonatal birthweight at term ( $P = .011$ ).

**Conclusion:** Cardiac biomarkers have an important role for investigating suspected disease in high-risk pregnant women, but a robust assessment of the levels expected in healthy pregnant women is an essential prerequisite to their application in clinical practice. This study has

defined trimester- and BMI-specific reference intervals for NT-pro BNP and BNP, which may improve how women with suspected cardiovascular disease are investigated in pregnancy.

**Key Words:** Pregnancy, NT-pro BNP, BNP, natriuretic peptide, reference interval, range

Cardiac disease is the leading cause of maternal mortality in the United Kingdom, constituting a quarter of deaths in pregnant women [1]. Cardiac biomarkers like troponin and natriuretic peptides are widely used to investigate suspected myocardial infarction [2] and heart failure [3] in nonpregnant adults, respectively. However, although these tests are commonly used in pregnancy, currently only general adult, nonpregnant reference limits are used, since pregnancy-specific ranges have not been defined.

The hormone B-type natriuretic peptide (BNP) and its inactive N-terminal fragment (NT-pro BNP) are released in response to cardiac strain and are widely used to investigate suspected heart failure [4, 5]. In the United States both biomarkers are recommended, but specific cut-offs are not supplied [5]. European guidelines suggest upper reference limits of 35 pg/mL for BNP and 125 pg/mL for NT-pro BNP to guide further investigation [4]. In contrast in the UK, NICE (National Institute for Clinical Excellence) guidelines recommend measuring NT-pro BNP only: low levels (<400 pg/mL) are rarely seen in cardiomyopathy, but very high levels (>2000 pg/mL) are strongly associated with adverse outcomes from myocardial disease [3]. Importantly, natriuretic peptide results vary substantially according to age, sex, and analytical method [6-8].

Despite the reliance on laboratory tests in modern high-risk obstetric care, reference intervals (RIs) for most investigations used in pregnancy (including BNP and NT-pro BNP) are extrapolated from nonpregnant populations. The physiological changes of pregnancy may alter the concentrations of natriuretic peptides [9], as pregnancy is associated with a 50% increase in cardiac output and ventricular remodeling, with marked, progressive plasma expansion [10], and an increase in glomerular filtration rate of at least 50% [11]. Levels of NT-pro BNP may be as low as 39 to 81 pg/mL in healthy women of childbearing age [12, 13], but this situation is more complex in women with disorders of pregnancy such as pre-eclampsia (a common obstetric condition associated with cardiac strain and renal impairment), where levels are reported to increase [14-16].

This study aimed to define RIs for BNP and NT-pro BNP in each trimester of uncomplicated pregnancy, in response to the previously highlighted unmet need [17]. Where these RIs differed from nonpregnant levels we investigated the potential underlying mechanisms by drawing comparisons to gestational changes in other commonly used biomarkers.

## Materials and Methods

The Oxford Pregnancy Biobank (REC 07/H0607/74) was searched for healthy pregnant women with stored EDTA plasma samples from each trimester (10-14<sup>+6</sup>, 18-23<sup>+6</sup>, and 31-38<sup>+6</sup> weeks). Venous blood samples were drawn by a research midwife upon recruitment and at each follow-up visit, and they were immediately centrifuged and stored at -80°C, a storage condition previously shown to stabilize the natriuretic peptides. Inclusion criteria were adult pregnant women with uncomplicated pregnancies who delivered at term. Exclusion criteria were women with pre-existing hypertension or diabetes, or women who developed gestational hypertension or diabetes, pre-eclampsia, HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets), acute fatty liver of pregnancy, obstetric cholestasis, or preterm delivery (<37 completed weeks). The sample size was determined by the number of eligible participants in the biobank, and we performed a post hoc power calculation based on the results.

Plasma BNP and NT-pro BNP were measured at John Radcliffe Hospital, Oxford, using an immunoturbidimetric method on the Abbott Architect i2000 analyzer (Abbott Laboratories Ltd, Maidenhead, UK) after a single freeze-thaw cycle. Further details are available from the Antibody Registry regarding the specific antibodies used to measure BNP [18] and NT-pro BNP [19]. The between-batch variability for BNP, as measured in our laboratory and expressed as the coefficient of variation (CV)%, was 4.7% at 79 pg/mL, 7.2% at 345 pg/mL, and 10.2% at 1596 pg/mL. Comparatively, imprecision for NT-pro BNP was 5.6% at 7.9 pg/mL, 4.3% at 43.5 pg/mL, and 3.8% at 365 pg/mL. The lower limits of detection were 10 pg/mL and 8 pg/mL, respectively.

RIs were calculated in accordance with IFCC (International Federation of Clinical Chemistry) guidelines [20] using similar methods to a previous paper from this group in which we defined pregnancy-specific RIs [21]. Data on NT-pro BNP were approximated to a normal distribution in each trimester using a logarithmic transformation. Participants were binned into equal groups of maternal age and body mass index (BMI) for each biomarker, as performed in other studies of RIs [22], and outliers were identified and excluded within these bins using Horn's method [23]. BNP could not be transformed so outliers were identified by visually examining the distribution. There is no

consensus on how to manage results that fall below the lower detection limit, but it is widely accepted that, if this constitutes a large proportion of the results, excluding them would introduce systematic bias [24]. As BNP was unmeasurably low in 27% of all women, we included all such results, allocated each an equal arbitrary value of a lesser value than the lowest measurable result, and defined RIs using a nonparametric method based on ranks rather than values, as per IFCC guidance [20]. RIs were estimated as the 2.5th and 97.5th centiles and were presented with their respective 90% CIs [20]. We performed a sensitivity analysis to investigate the effect on RIs for NT-pro BNP when using untransformed data, as for BNP.

Subgroup analyses were performed using Student's t-tests, Wilcoxon Rank Sum tests, or chi-squared tests, according to groups of maternal age, BMI at booking (recorded in the first trimester), mean arterial pressure at booking, gestation at delivery, and fetal sex. Multivariate linear regression models were used to investigate the associations between NT-pro BNP and BMI, and neonatal birthweight. We then used fractional polynomial regression to model the 2.5th, 50th, and 97.5th centiles for NT-pro BNP for any given value of BMI, using the third trimester as an example. The chosen model was that which most accurately predicted NT-pro BNP using combinations of powers for BMI (from -3, -2, -1, -0.5, 0, 0.5, 1, 2, and 3), and the standard deviation was estimated by regressing the residuals as a linear function of BMI.

To investigate changes in NT-pro BNP and BNP according to gestational age, we compared the rates of change between trimesters with those seen in other biomarkers that are well described in pregnancy. Hemoglobin and platelets were selected to reflect parameters whose longitudinal changes in concentration during pregnancy are thought to be (in part) due to hemodilution. Levels of hemoglobin (g/dL) and platelet count ( $10^9/L$ ) were extracted from the 30 women who had these results available in all 3 trimesters, as part of their routine antenatal care,

and these were compared with the concurrent natriuretic peptides at each time point. Partitioning was investigated using Lahti's method [25]. Summary statistics were presented as the mean and standard deviation or frequencies with proportions, as appropriate. Statistical significance was assumed where  $P < .05$ , or  $P < .01$  for subgroup analyses. All analyses were performed using Stata (version 16.1, 2021).

## Results

### Participant Characteristics

For NT-pro BNP, a total of 264 women were identified from the biobank, of whom 4 (1.5%) were excluded as outliers in the first trimester only (12.0, 12.2, 14.2, and 378 pg/mL). Of the 260 women included, 94% were white, and the mean BMI and mean arterial pressure were 25 kg/m<sup>2</sup> and 79 mmHg, respectively. Most babies were born at (or after) full term (mean gestation 40.4 weeks) and fewer than 2% of babies required admission to the neonatal unit. Only 6 women (2.3%) had any missing data. Compared with those with a normal BMI, overweight women had a higher booking blood pressure, delivered later and had larger babies. Data on participant characteristics are presented in Table 1. For BNP, a total of 249 women were eligible, and no outliers were identified.

### NT-pro BNP

Mean levels of NT-pro BNP were similar in the first and second trimesters ( $P_{diff} = .164$ ) but significantly lower in the third trimester ( $P_{diff} < .001$ ) (see Fig. 1). This corresponds with a joint upper reference limit for the first 2 trimesters (<200 pg/mL) and a substantially lower limit in the third trimester (<150 pg/mL) (see Table 2). NT-pro BNP was not associated with maternal age, fetal sex, or gestation at delivery

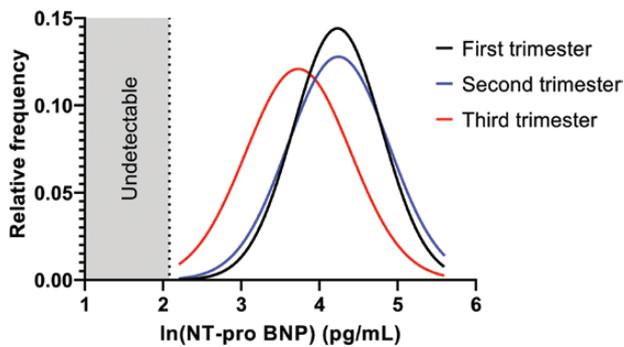
**Table 1.** Participant characteristics

Variable	All women	Body mass index		$P_{diff}^a$	Missing (n, %)
		<25 kg/m <sup>2</sup>	≥25 kg/m <sup>2</sup>		
Age (years)	30.1 (5.0)	30.2 (5.0)	30.1 (4.9)	.84	—
Body mass index (kg/m <sup>2</sup> )	25.0 (4.3)	22.2 (1.7)	29.1 (3.4)	—	—
Mean arterial pressure (mmHg)	79.1 (7.8)	77.0 (7.4)	82.2 (7.2)	<.001	4 (1.5)
Ethnicity (n white, % of total)	243 (94.2%)	143 (94.7%)	100 (93.5%)	.684	2 (0.8)
Gestation at delivery (weeks)	40.4 (1.1)	40.3 (1.2)	40.5 (1.0)	.047	—
Neonatal birthweight (kg)	3.5 (0.5)	3.4 (0.5)	3.6 (0.5)	<.01	—
Neonatal admissions (n, %)	5 (1.9)	4 (2.6%)	1 (0.9%)	.92	—
Fetal male sex (n, %)	129 (49.6)	76 (49.7%)	53 (49.5%)	.99	—

Values shown are means with standard deviations, or numbers and proportions.

Data on age, body mass index and mean arterial pressure were taken at recruitment.

<sup>a</sup>Calculated by Student's t-tests or comparison of proportions.



**Figure 1.** Smooth distribution curves of NT-pro BNP.

(see Table 3). It was negatively associated with BMI from the second trimester onwards (Fig. 2) but partitioning of the RI by BMI was only justified in the third trimester (see Table 4). Mean arterial pressure was not associated with NT-pro BNP after adjustment for BMI. Interestingly, NT-pro BNP in the first trimester was inversely, linearly associated with neonatal birthweight at term ( $P = .011$ ), which remained significant after adjustment for first trimester BMI and blood pressure ( $P = .016$ ) (See Fig. 3). A sensitivity analysis showed that the RIs were not materially different when outliers were identified visually and RIs were based on untransformed data (RI <187, 192, and 153 pg/mL in the first, second and third trimesters, respectively).

### B-type Natriuretic Peptide

The upper reference limits for BNP were similar in each trimester (50 pg/mL). There were no significant associations with midtrimester BNP and maternal age ( $P = .32$ ), fetal sex ( $P = .59$ ), gestation at delivery ( $P = .55$ ), or BMI ( $P = .54$ ). The median BNP was marginally lower in women with a mean arterial blood pressure over 80 mmHg (5.2 pg/mL), but this was not significant at the 1% level ( $P = .011$ ). Unlike NT-pro BNP, BNP was similar in babies that were small, large, or appropriate for gestational age (Kruskal-Wallis test,  $P = .42$ ).

### Rates of Change and Other Markers

Median levels of hemoglobin dropped between the first and second trimesters and then stabilized, and platelets fell consistently with each trimester. In contrast, both NT-pro BNP and BNP did not decrease in concentration until late pregnancy. Absolute and relative trimester-specific changes for each biomarker are presented in Figs. 4 and 5, respectively. While the upper reference limit for BNP was stable, the median levels in late pregnancy dropped more substantially, relative to the change in NT-pro BNP. Lastly, the smoothed RI for NT-pro BNP is expressed as a continuous function

of BMI in Fig. 6, where NT-pro BNP was most simply and accurately predicted using a linear model with a natural logarithmic transformation.

## Discussion

This is the largest known study to date examining NT-pro BNP and BNP in healthy pregnant women with the explicit objective of deriving pregnancy-specific RIs. In each trimester, the upper limits for NT-pro BNP were lower than the clinical limit recommended by NICE for heart failure in nonpregnant adults [3], but they were higher than the nonpregnant cut-off in European guidelines [4]. There was a significant reduction in NT-pro BNP by late pregnancy, and the overall upper reference limit was 25% lower by the third trimester. This was even lower in association with a high BMI, in keeping with previous studies of natriuretic peptides [26]. A BMI-specific RI for NT-pro BNP was statistically justified for use in overweight women, which will require further clinical evaluation. Natriuretic peptides are not routinely interpreted according to partitioned RIs outside of pregnancy, so this is a significant new pregnancy-specific finding, which may enable a more nuanced approach in pregnant women.

While previous, smaller studies have reported stable levels of NT-pro BNP throughout pregnancy [17, 27], our study is consistent with the findings of Blohm et al., who reported lower levels at term [28]. BNP was comparatively consistent across different gestational ages and it did not require trimester-specific adjustment, nor was it associated with BMI or birthweight. The observation that NT-pro BNP and BNP demonstrate different trends is unsurprising. Although they are produced concurrently at equimolar concentrations, their pathophysiological characteristics and analysis are distinctly different [29]. For example, although the half-life of the biologically inactive NT-pro BNP is over 60 minutes, the active hormone BNP is between 15 and 20 minutes. Importantly, BNP degradation is significantly influenced by the circulating activity of the metalloprotease neprilysin [30]. Our group has recently reported that the placenta releases active neprilysin into the maternal circulation on syncytiotrophoblast-derived extracellular vesicles [31]. This is highly likely to influence the half-life and measurable concentration of BNP, but not NT-pro BNP, during pregnancy. Consequently, NT-pro BNP may be a more robust marker than BNP in pregnancy.

In healthy individuals, age, sex, and BMI are well-defined characteristics that influence NT-pro BNP, and in one large study these accounted for 33% of interindividual variation [32]. The study noted that in nonpregnant women

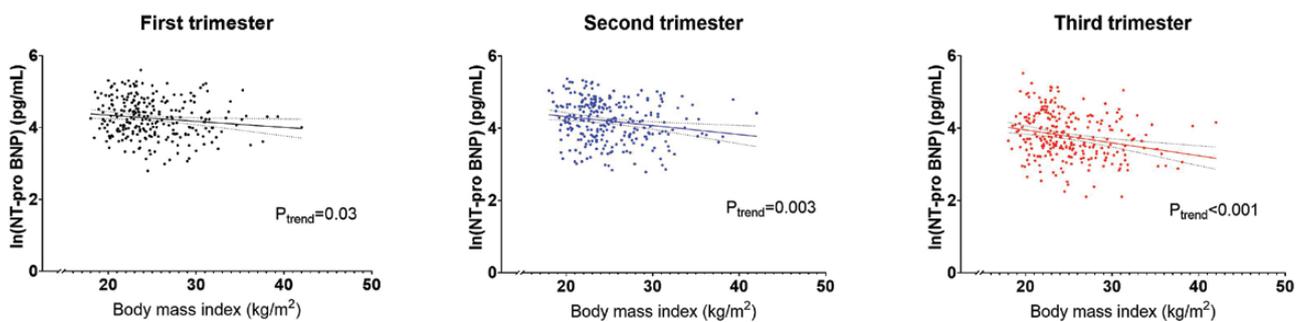
**Table 2.** Trimester-specific reference intervals

Biomarker	Trimester	Median	Lower reference limit	Upper reference limit	Suggested reference interval
		(pg/mL)	(pg/mL)	(pg/mL)	(pg/mL)
NT-pro BNP	First	68.8	24.6 (21.5-28.2)	187.6 (182.0-200.6)	<200
	Second	68.0	20.1 (17.7-23.5)	191.9 (165.6-198.2)	
	Third	40.6	12.6 (9.6-16.2)	155.1 (142.1-171.0)	
BNP	First	16.5	<10	47.0 (40.8-51.8)	<50
	Second	17.1		50.9 (47.1-59.8)	
	Third	12.2		51.5 (41.9-65.7)	

**Table 3.** NT-pro BNP by participant characteristics

Characteristic	n	First trimester		Second trimester		Third trimester		
		Mean	$P_{diff}$	Mean	$P_{diff}$	Mean	$P_{diff}$	
Maternal age	≤30 years	129	70.0	.74	67.1	.84	43.0	.80
	>30 years	131	71.6		66.2		43.8	
Body mass index	<25 kg/m <sup>2</sup>	153	74.9	.04	72.4	.006	49.4	.0001
	≥25 kg/m <sup>2</sup>	107	65.4		59.2		36.1	
Mean arterial pressure	<80 mmHg	142	75.7	.014	73.5	.002 <sup>a</sup>	48.4	.001 <sup>a</sup>
	≥80 mmHg	114	64.3		58.6		37.4	
Fetal sex	Male	129	70.3	.82	65.5	.62	42.7	.67
	Female	131	71.3		67.8		44.1	
Gestation at delivery	≤40 weeks	91	73.4	.43	70.6	.24	45.3	.44
	>40 weeks	169	69.5		64.6		42.4	

<sup>a</sup>Not significant after adjustment for body mass index.

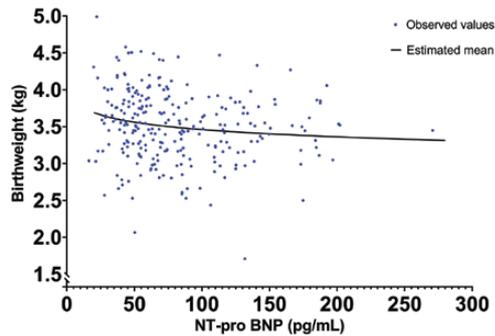
**Figure 2.** NT-pro BNP by body mass index. Observed values and linear estimates.

aged 25-29 years, the 97.5th centile of NT-pro BNP was 153.7 pg/mL, which is lower than the upper reference limit that we report in the first and second trimester (<200 pg/mL), but similar to the third trimester (<150 pg/mL). This suggests that the higher NT-pro BNP concentrations we report are pregnancy related.

There are several potential mechanisms underlying the dynamic changes in natriuretic peptides during pregnancy. Yoshimura et al. reported increased levels of BNP in response to cardiac strain [33], whereas it is widely accepted that other markers, including hemoglobin and platelets, are hemodiluted as part of the normal

**Table 4.** Partitioned reference intervals for NT-pro BNP

	BMI group	Lower limit	Upper limit	$D_L$	$D_U$	Partitioning	Reference interval
		(pg/mL)	(pg/mL)				(pg/mL)
First trimester	<25 kg/m <sup>2</sup>	27.4	197.5	0.42	0.16	No	<200
	≥25 kg/m <sup>2</sup>	31.7	181.6				
Second trimester	<25 kg/m <sup>2</sup>	21.5	197.0	0.41	0.50	No	
	≥25 kg/m <sup>2</sup>	17.4	151.7				
Third trimester	<25 kg/m <sup>2</sup>	12.7	169.5	0.47	0.79	Yes	<170
	≥25 kg/m <sup>2</sup>	9.9	112.1				<110

**Figure 3.** First trimester NT-pro BNP and neonatal birthweight. Observed values and linear estimate.

physiological changes of pregnancy. In our study, overall levels of NT-pro BNP and BNP remained static in the first half of pregnancy (in some cases rising), before subsequently falling by the third trimester. This effect was differentially modified by BMI, which acts as a proxy for the degree of plasma expansion [34]. This finding is in keeping with smaller previous studies [35] and supports the hypothesis that absolute levels of natriuretic peptides are elevated during pregnancy, but the progressive effect of hemodilution eventually supersedes this. As the mechanism driving the initial elevation is likely to be increased cardiac strain [36], we propose that these markers could have a meaningful role in managing pregnancy-related cardiovascular diseases, using the appropriate reference limits, which we have now defined.

Studies have reported elevated levels of NT-pro BNP in established pre-eclampsia with or without ventricular failure [14, 16, 37], but the benefit of using NT-pro BNP for predicting pre-eclampsia is uncertain [38, 39]. A recent study reported a better predictive value for pre-eclampsia when adding NT-pro BNP to the existing biomarkers (soluble FMS-like tyrosine kinase 1 and placental growth factor) [40]. However, the NT-pro BNP cut-off used (174 pg/mL) falls directly between the 2 reference limits defined in our study, and we propose that our findings in support of trimester-specific RIs may improve this further.

Our study also found that higher levels of NT-pro BNP in early pregnancy were associated with a lower neonatal birthweight at term. This is consistent with a 2016 study by Sadlecki et al. [39] but is contrary to the findings of others [17, 41]. The potential for using NT-pro BNP alongside other markers to predict fetal growth restriction should be investigated, particularly considering the emerging evidence for the roles of neprilysin and other markers associated with placental function.

### Strengths and Limitations

The study population was predominantly white so we could not compare differences between ethnic groups. The inclusion criteria were healthy pregnant women, so it is beyond the scope of this study to comment on the performance of BNP or NT-pro BNP for detecting disease, although defining an accurate RI is an essential prerequisite for using biomarkers in clinical practice [42].

This study used data on BMI collected in the first trimester. We acknowledge that BMI increases variably during pregnancy in association with fetal weight gain and amniotic fluid volume [43]. Current guidelines support the use of pre- or early-pregnancy BMI measurements in other areas of obstetric practice [44], and we used this as a pragmatic way of investigating the effect of BMI. The distributions of data on BNP and NT-pro BNP were different and required methodological changes to address this. While has the potential to modify the results, a sensitivity analysis showed that is unlikely to have a material effect in clinical practice.

### Conclusions

The RI for NT-pro BNP changes significantly throughout the course of pregnancy, likely because of the interplay between cardiac strain and hemodilution. BNP shows similar trends, but it is difficult to assess its response to the underlying physiological changes. Pregnancy-specific upper

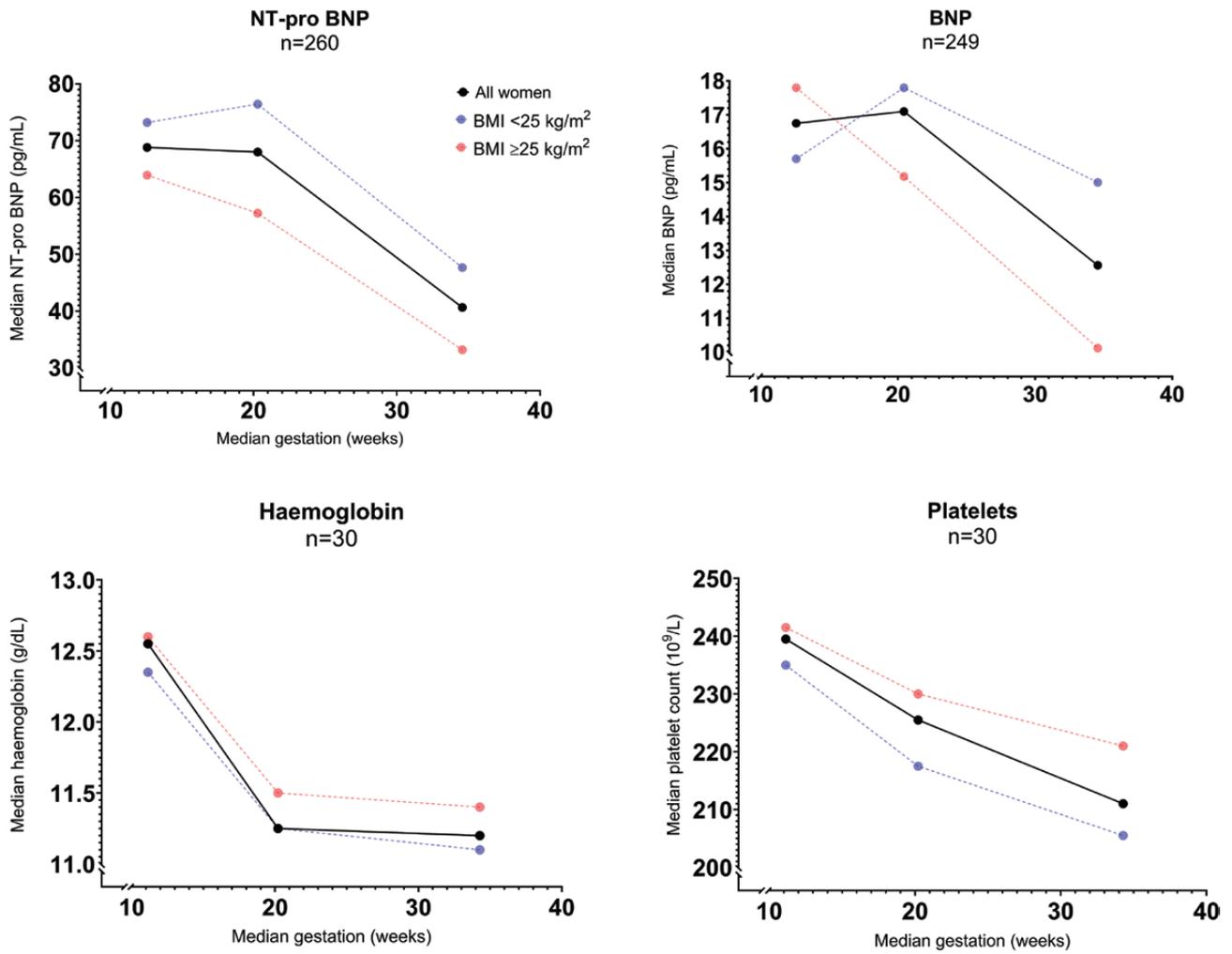


Figure 4. Comparative biomarker concentrations by trimester and body mass index.

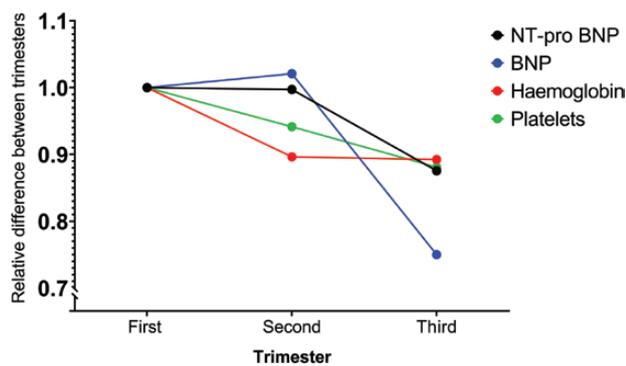


Figure 5. Comparative rates of change by trimester.

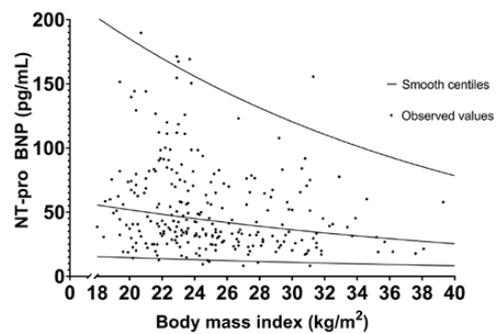


Figure 6. Smoothed reference limits for NT-pro BNP by body mass index.

reference limits for both natriuretic peptides have potential benefits for identifying pregnant women with cardiovascular disease, pre-eclampsia, and predicting intrauterine growth restriction.

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## Additional Information

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**Disclosures:** The authors do not declare any conflicts of interest.

**Data Availability:** Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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