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RESEARCH ARTICLE

Arterial stiffness and blood pressure are similar in naturally menstruating and oral contraceptive pill-using women during the higher hormone phases

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Abstract

Elevations in blood pressure (BP) are understood as having a bidirectional relationship with stiffening of central and peripheral arteries. Arterial stiffness is mitigated by oestrogen, which aides in arterial vasorelaxation. To evaluate whether BP, stiffness, and pressure waveforms were different between young healthy naturally menstruating (non-OCP) and oral contraceptive pill (OCP)-using women, we measured brachial and aortic BPs, carotid-to-femoral pulse wave velocity, carotid β-stiffness, elastic modulus, central augmentation index and augmentation index normalized to a heart rate of 75 bpm, and forward and backward pressure waveforms in 22 women (22 (1) years, OCP: $n = 12$). To assess phasic differences, women were studied during the early follicular (≤5 days of menstruation onset) and early luteal (4 (2) days post-ovulation) phases of non-OCP and compared to the placebo pill (≤5 days of onset) and active pill (≤5 days of highest-dose active pill) phases of OCP. During the lower hormone phases, OCP users had significantly higher brachial systolic blood pressure (SBP) (119.3 (8.3) vs. 110.2 (8.3) mmHg, $P = 0.02$) and aortic SBP (104.10 (7.44) vs. 96.80 (6.39) mmHg, $P = 0.03$) as compared to non-OCP users; however, during the higher hormone phases, there were no differences in measures of brachial or aortic BP, arterial stiffness, or indices of BP waveforms between OCP and non-OCP users ($P \geq 0.05$). In conclusion, exogenous and endogenous hormones have similar influences on BP and arterial stiffness; however, lower levels of exogenous hormones augment both central and peripheral BPs.

KEYWORDS
arterial stiffness, blood pressure, OCP, oestrogen, premenopausal, pulse wave velocity

1 | INTRODUCTION

Stiffening of the arterial walls is a hallmark mechanism in the onset and progression of many common cardiovascular diseases. Structurally, arterial stiffness is characterized by medial layer disorganization and/or loss of the more pliable elastin fibres, with concomitant accumulation of the more rigid collagen fibres (Palombo & Kozakova, 2016). The relationship between arterial stiffness and blood pressure (BP) is currently understood as being bidirectional, whereby increased stiffening of arterial walls can heighten systemic BP, and inversely, an increase in the vascular distension pressure can elicit increases in arterial stiffness (Fleenor & Berrones, 2015). However, arterial stiffening is thought to occur prior to the manifestation of hypertension (Vlachopoulos et al., 2015). Specifically, increases in arterial stiffness

enhance the transmission velocity of both the forward traveling pressure (P_f) wave and the reflected pressure (P_b) wave, causing the P_b wave to occur earlier at the site of the central aorta (Vyas et al., 2007). Early arrival of the P_b wave leads to augmented amplitude and duration of the pressure wave, causing increases in aortic BP (Vyas et al., 2007). Therefore, measures of arterial stiffness and indices of pressure waveforms (P_f and P_b) can be useful in the early recognition and risk stratification for cardiovascular diseases (Vlachopoulos et al., 2015).

While some groups have reported variations in measures of arterial stiffness across the menstrual cycle (Giannattasio et al., 1999; Hayashi et al., 2006; Williams et al., 2001), others report no significant changes (Ounis-Skali et al., 2006; Priest et al., 2018; Yu et al., 2014). Additionally, some studies have revealed an effect of exogenous hormones, via oral contraceptive pill (OCP) use, on measures of arterial stiffness and/or central and peripheral haemodynamics (DuPont et al., 2019; Hickson et al., 2011; Seeland et al., 2020; Yu et al., 2014). While fluctuating concentrations of oestrogen and progesterone occurring across the natural menstrual cycles of premenopausal women are understood to generally influence haemodynamics, arterial stiffness, and indices of the pressure waveform, the specific hormone phase-dependent effects on these parameters remain controversial. Furthermore, there is a lack of data regarding measures of BP, arterial stiffness and indices of the pressure waveform, especially during the early luteal (EL) phase when concentrations of progesterone are rising in the presence of oestrogen concentrations falling past their initial peak (post-ovulation). Therefore, the current study investigates the effects of natural and exogenous hormone fluctuations during different phases of the menstrual (early follicular, EF; and early luteal, EL) and OCP (placebo pill and active pill) cycle.

Specifically, the aim of this study was to evaluate differences in measures of brachial and aortic BP, central (carotid-to-femoral pulse-wave velocity (cfPWV)) and local (β-stiffness and pressure–strain elastic modulus (E_p)) arterial stiffness, and indices of pressure waveforms (augmentation index (AIx), AIx normalized to a HR of 75 bpm (AIx_{75}), P_f and P_b) between young healthy, naturally menstruating (non-OCP) and OCP-using women. Further, we sought to assess within-group differences among the aforementioned measures between the lower and higher oestrogen and progesterone phases of OCP and non-OCP cycles. We hypothesized that young healthy OCP- and non-OCP-using women would have higher central (aortic) and peripheral (brachial) BPs during the lower hormone phases as compared to the higher hormone phases. Between groups, we hypothesized that OCP users would have higher central and peripheral BPs only during the placebo pill (PP) phase as compared to non-OCP users during the EF phase. Further, we hypothesized that among OCP and non-OCP users, measures of both central (cfPWV) and local (β-stiffness and E_p) arterial stiffness as well as indices of the pressure waveform (AIx, AIx_{75}, P_f and P_b) would be increased during the lower hormone phases as compared to the higher hormone phases. Lastly, we hypothesized that OCP users would have higher central and local arterial stiffness and indices of the pressure waveform only during the PP phase as compared to non-OCP users during the EF phase.

New Findings

- **What is the central question of this study?**
  Are there differences in blood pressure, arterial stiffness and indices of pressure waveforms between young oral contraceptive pill-using and naturally menstruating women during lower and higher hormone phases of their cycles?
- **What is the main finding and its importance?**
  Blood pressure, arterial stiffness and indices of pressure waveforms are influenced similarly by exogenous and endogenous hormones. However, lower levels of exogenous hormones moderately increase blood pressure among oral contraceptive pill-using women.

2 | METHODS

2.1 | Ethical approval

This study's protocol conformed to the most recent provisions of the *Declaration of Helsinki* (except for registration in a database) and was approved by the University of Maryland, Institutional Review Board on 2 October 2018 (Reference number: 1290733). All participants provided written informed consent.

2.2 | Participants

We recruited individuals in and around the University of Maryland, College Park who self-reported as conforming to the following initial inclusion criteria: (1) female sex; (2) between 18 and 39 years of age; (3) body mass index (BMI) <30 kg/m^2; (4) resting BP <140/90 mmHg; and (5) non-users of tobacco or nicotine products. Twenty-five women met the initial inclusion criteria to participate, of whom 22 completed the study. On Visit 1, participants provided written informed consent then completed a more detailed health history questionnaire to confirm no present or past cardiac, pulmonary or metabolic conditions, cancer, stroke, epilepsy, deep vein thrombosis, arthritis, pain or dizziness with exercise, and/or use of insulin, anti-anginal, antihypertensive or thyroid medications, and/or anticoagulation therapy. If a participant reported presence or history with one of the above conditions, they were excluded from further participation in the present study. Additionally, to meet the inclusion parameters, participants confirmed that they either (1) consistently used the same brand and formulation of OCPs, or (2) experienced natural, regular menstruation for (at minimum) the past 6 months. Regular menstruation was defined with the following parameters: regularity of menses with variance of ±4 days, frequency of menses occurring every 24–38 days, and duration of

≤8 days of menstrual flow (Munro et al., 2018). All eligible women also reported consistency in their menstrual or pill cycles, lack of amenorrhea, and no possibility of pregnancy for (at minimum) the past 6 months. All participants confirmed that they had not used one-time emergency contraceptive pills, intrauterine devices, hormonal patches, shots, vaginal rings, or implants containing synthetic oestrogen or progesterone for at least 6 months prior to the start of the study.

2.3 | Study procedures

Participants reported to the Human Integrative Physiology Laboratory at The University of Maryland, College Park for three separate visits. Participants were instructed to refrain from exercise and alcohol consumption for ≥12 h and from non-regular medications (including non-steroidal anti-inflammatory drugs) for ≥24 h before all study visits. Prior to Visit 1, participants were requested to fast from all food and drink (other than water) for ≥4 h, whereas prior to Visits 2 and 3, participants were requested to fast for ≥12 h. For Visit 1, timing/phase of the OCP or menstrual cycle was not controlled for.

Visit 2 occurred during the lower hormone phases of women; these were defined as the placebo pill (PP) phase for OCP participants and the EF phase for non-OCP participants. Visit 2 was scheduled ≤5 days from OCP participants having started their placebo-pills (i.e., withdrawal bleeding), or ≤5 days from the start of menstruation (i.e., menstrual bleeding) for non-OCP participants. Visit 3 occurred during the higher hormone phase, which was defined as the active pill (AP) and early luteal (EL) phases for OCP users and non-OCP participants, respectively. For OCP users, Visit 3 was scheduled during the week of their OCP pack when participants were taking the highest doses of exogenous oestrogen and progesterone. To schedule Visit 3 for non-OCP participants, the EL phase was determined using an ovulation kit (Clearblue Advanced Digital Ovulation Test, Swiss Precision Diagnostics GmbH, Geneva, Switzerland). Non-OCP participants were instructed to alert researchers as soon as a positive ovulation icon appeared on the screen of the ovulation kit. Visit 3 was then scheduled within an average of 4 (2) days from the positive ovulation test. For all participants, Visit 3 was scheduled within an average of 20 (7) days from Visit 2.

2.4 | Resting haemodynamics and anthropometrics

Upon arrival to the laboratory for Visit 1, participants rested seated for ≥10 min before BP was measured twice on the right arm using an automated oscillometric device (Omron Corporation, Kyoto, Japan). If the two sequential BP values differed by ≥5 mmHg, a third measurement was taken. The average of the two closest BP values was recorded. Resting heart rate (HR) was measured using a HR monitor (Polar Electro, Woodbury, NY, USA). Participants' height was measured using a stadiometer and weight with a beam-balanced scale. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared.

2.5 | $\dot{V}_{O_2peak}$ exercise test

Following haemodynamic and anthropometric measures, cardiovascular health and fitness were assessed with a cycle ergometer $\dot{V}_{O_2peak}$ exercise test, as previously described (Eagan et al., 2021). Briefly, $\dot{V}_{O_2peak}$ was determined using a graded exercise protocol lasting until participants met volitional fatigue or failed to maintain a cycling cadence of 75–80 rpm. Measures of exercising HR, brachial BP and ratings of perceived exertion were collected in the last 15 s of each 2-min stage. Continuous oxygen consumption was measured with a breath-by-breath metabolic system (Parvo Medics, Salt Lake City, UT, USA).

2.6 | Blood collection for analyses of oestrogen and progesterone concentrations

Upon arrival for Visits 2 and 3, blood was drawn from an antecubital vein into serum separator tubes, spun, pipetted and stored in samples as described in a previous manuscript (Eagan et al., 2021). Serum samples were comprehensively analysed using enzyme-linked immunoassays to measure concentrations of endogenous oestrogen and progesterone, respectively. Procedures for obtaining hormone concentrations are described with greater detail in the previous manuscript (Eagan et al., 2021).

2.7 | Central and peripheral blood pressure

Following the blood draw and at least 10 min of supine rest, BP waveforms were obtained from participants using an oscillometric cuff placed around the right brachial artery (Sphygmocor XCEL, AtCor Medical, Sydney, Australia). The SphygmoCor XCEL software's proprietarily designed and validated computerized transfer function reconstructs the peripheral BP waveforms into central BP waveforms – providing measures of brachial and aortic systolic (bSBP, aSBP), diastolic (bDBP, aDBP) and mean arterial (bMAP, aMAP) pressures, respectively.

2.8 | Indices of pressure waveforms

The augmentation index (AIx), defined as the difference between the first and the second systolic peak of the central BP wave (P_2 – P_1), was calculated by SphygmoCor XCEL software and expressed as a percentage of the pulse pressure based on the propagation time delay of the reflected wave (as in Equation. 1). While measures of AIx are contingent on the stiffness of the large arteries, they are also influenced by peripheral resistance in the medium and small vessels, and can be affected by parameters such as the participant's resting HR (Papaioannou et al., 2005, 2019). Thus, an AIx normalized to a HR of 75 bpm (AIx_{75}) was used to account for potential differences in HR between participants. To determine the aortic forward

(P_f) and backward (P_b) pressure waveform components, BP wave separation analyses were also performed by the SphygmoCor XCEL software.

$$\text{AIx}\,(\%) = \frac{(P_2 - P_1)}{(\text{SBP} - \text{DBP})} \times 100 \quad (1)$$

2.9 | Central arterial stiffness

Central arterial stiffness was assessed via carotid-to-femoral PWV (cfPWV). Two different pulse waves were recorded sequentially by applanation tonometry; the first was measured at the pulse of the right common carotid artery and the second was measured at the pulse of the femoral artery. The distance travelled by the pressure waves between the two sensors was estimated using a tape measure; distances (m) between participants' suprasternal notch to the pulse of their femoral artery, the pulse of their carotid artery to their suprasternal notch, and from the site of the femoral pulse to the placement of the thigh cuff were obtained and inputted into the SphygmoCor XCEL software. Transit time of the pressure wave from the carotid artery to the femoral artery was determined via automatic detection of the diastolic foot at each pressure wave. cfPWV was calculated by the SphygmoCor XCEL equipment:

$$\text{cfPWV} = \frac{\text{Distance (m)}}{\text{Transit Time (s)}} \quad (2)$$

2.10 | Local arterial stiffness

Local arterial stiffness was evaluated with a high-definition echo-tracking ultrasound system equipped with a 7.5-MHz linear probe placed ~10 cm proximal to the bifurcation of the right common carotid artery (Alpha 10, Hitachi Aloka Medical, Mitaka-Shi, Tokyo, Japan). Echo-tracking gates were manually set to read at the boundaries between the tunica intima and tunica media of the anterior and posterior carotid arterial walls. The carotid arterial pressure-to-diameter relationship is thought to be linear (Sugawara et al., 2000). Thus, measures of brachial BP were utilized for the calculation of indices of carotid arterial stiffness (β-stiffness) and the pressure–strain elastic modulus (E_p). At least six consecutive beats were recorded and averaged to obtain a representative waveform. The following indices were calculated (as in Equations 3 and 4), where P_s and P_d are carotid arterial systolic and diastolic BPs, and D_s and D_d are carotid arterial systolic and diastolic diameters, respectively:

$$\beta\text{Stiffness (AU)} = \ln\left(\frac{P_s}{P_d}\right) \times \left(\frac{D_s - D_d}{D_d}\right) \quad (3)$$

$$E_p\,(\text{kPa}) = \frac{(P_s - P_d)}{\left(\frac{D_s - D_d}{D_d}\right)} \quad (4)$$

2.11 | Statistical analyses

Data from all variables are reported as means (SD). Normality was assessed using the Shapiro–Wilk test and outliers were identified using the ROUT method (Q = 1%). An unpaired Student's t-test was used to compare baseline descriptive characteristics between OCP and non-OCP users from Visit 1. Two-way repeated measures analysis of variance (ANOVA) was used to assess the effects of group (OCP vs. non-OCP), phase (PP and EF vs. AP and EL), and group × phase interaction effects on measures of aortic and brachial SBP, DBP and MAP, cfPWV, β-stiffness, E_p, AIx, AIx_{75}, P_f and P_b. In the event of a significant interaction effect or significant effect of group detected on a variable, a planned comparisons *post hoc* Bonferoni's multiple comparisons test was performed to determine whether the difference between OCP and non-OCP users was occurring during the lower hormone (PP vs. EF) and/or higher hormone (AP vs. EL) phases. Similarly, in the event of a significant interaction effect or significant effect of phase detected on a variable, a planned comparison *post hoc* Bonferoni's multiple comparisons test was performed to determine whether the difference between phases was occurring between the lower and higher hormone phases of OCP (PP vs. AP) and/or of non-OCP (EF vs. EL) use. The significance level for all analyses was set at a P-value of <0.05. Magnitudes of comparisons were assessed by calculating corrected effect sizes (Hedges's g), as in:

$$\text{Hedges's } g = \left(t\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}\right) \times \left(1 - \frac{3}{4(n_1 + n_2) - 9}\right) \quad (5)$$

where t corresponds to the t-value and n corresponds to the dataset's sample size (Lakens, 2013). Effects were considered small when Hedges's $g \leq 0.2$, medium when Hedges's $g = 0.5$, and large when Hedges's $g \geq 0.8$ (Lakens, 2013). All statistical analyses were performed using GraphPad Prism (Version 8, GraphPad Software, San Diego, CA, USA), except for effect sizes.

3 | RESULTS

3.1 | Participant characteristics

A total of twenty-two participants (OCP, n = 12) completed the study. Baseline characteristics for young healthy OCP and non-OCP women are presented in Table 1. All participant characteristics from Visit 1 were statistically similar between OCP and non-OCP users except bDBP, which was significantly higher in OCP as compared to non-OCP users (P = 0.0196, Hedges's g = 1.05; Table 1). The number of OCP participants using first, second or third generation and monophasic, biphasic or triphasic pill formulations are provided in Table 2. No participants were using OCPs classified as fourth generation pills.

TABLE 1 Participant characteristics compared between OCP ($n = 12$) and non-OCP ($n = 10$) participants using independent *t*-tests

Characteristic	OCP ($n = 12$)	Non-OCP ($n = 10$)
Age (years)	22 (3)	22 (2)
BMI (kg/m^2)	23 (3)	24 (2)
Resting HR (bpm)	70 (11)	74 (8)
Resting bSBP (mmHg)	117 (9)	110 (6)
Resting bDBP (mmHg)	74 (10)	65 (6)*
$\dot{V}_{O_2peak}$ (ml/kg/min)	37 (6)	32 (7)

Data are means (SD). *$P < 0.05$. BMI, body mass index; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure

TABLE 2 Generation and formulation of OCPs used by participants

	Formulation			
Generation	Monophasic (*n*)	Biphasic (*n*)	Triphasic (*n*)	Total (*n*)
First (*n*)	5	0	0	5
Second (*n*)	2	1	0	4
Third (*n*)	2	1	1	3
Total (*n*)	9	2	1	12

First generation pills included norethindrone ($n = 5$), second generation pills included desogestrel ($n = 2$) or levonorgestrel ($n = 3$), and third generation pills included norgestimate ($n = 3$)

3.2 | Oestrogen and progesterone

Among OCP users, serum concentrations of oestrogen and progesterone were similar between the PP and AP phases (oestrogen: 57.12 (29.10) vs. 53.0 (28.8) pg/ml; progesterone: 1.4 (1.0) vs. 1.2 (0.9) ng/ml), as previously reported (Eagan et al., 2021). Contrastingly, among non-OCP users, serum concentrations of oestrogen and progesterone were significantly lower in the EF phase as compared to the EL phase (oestrogen: 91.8 (34.5) vs. 120.9 (32.6) pg/ml; progesterone: 1.4 (0.6) vs. 5.1 (3.6) ng/ml) (Eagan et al., 2021).

Between groups, concentrations of oestrogen are significantly lower among OCP users during the PP and AP phases as compared to non-OCP users during the EF and EL phases, respectively (Eagan et al., 2021). Concentrations of progesterone are also significantly lower among OCP users during the AP phase as compared to non-OCP users during the EL phase, but are statistically similar between groups during the PP and EF phases (Eagan et al., 2021).

3.3 | Central and peripheral blood pressure

There were significant group × phase interaction effects on bSBP, bMAP, aSBP, aDBP and aMAP (interaction effects: $P = 0.036$, 0.036, 0.008, 0.005 and 0.010, respectively, Table 3). There were significant effects of group on bSBP (main effect of group: $P = 0.043$), and significant effects of phase on bDBP, aDBP and aMAP (main effect of phase: $P = 0.041$, 0.005 and 0.037, respectively; Table 3).

During the lower hormone phases (PP vs. EF), OCP users had significantly higher bSBP and aSBP as compared to non-OCP users (bSBP: $P = 0.019$, Hedges's $g = 1.06$, Figure 1a; aSBP: $P = 0.021$, Hedges's $g = 1.01$, Figure 1c).

Among OCP participants, bDBP, bMAP, aSBP, aDBP and aMAP were significantly higher during the PP phase as compared to the AP phase (bDBP: $P = 0.017$, Hedges's g = 0.90, Figure 1b; bMAP: $P = 0.012$, Hedges's $g = 0.77$; aSBP: $P = 0.008$, Hedges's $g = 0.54$, Figure 1d; aDBP: $P = 0.0003$, Hedges's $g = 1.19$, Figure 1e; aMAP: $P = 0.003$, Hedges's $g = 0.85$, Figure 1f).

Among non-OCP participants, bSBP, bDBP, bMAP, aSBP, aDBP and aMAP were statistically similar between the EF and EL phases (bSBP: $P = 0.346$, Hedges's $g = 0.29$ bDBP: $P > 0.999$, Hedges's $g = 0.09$; bMAP: $P > 0.999$, Hedges's $g = 0.06$; aSBP: $P = 0.624$, Hedges's $g = 0.26$; aDBP: $P > 0.999$, Hedges's $g = 0.00$; aMAP: $P > 0.999$, Hedges's $g = 0.12$).

3.4 | Indices of pressure waveforms

There were no significant effects of group, phase or interaction on measures of AIx, AIx$_{75}$ or P_b (Table 3). Similarly, there were no effects of group or interaction on P_f (Table 3), although, there were significant effects of phase on measures of P_f ($P = 0.020$; Table 3). However, planned comparisons with *post hoc* Bonferoni's multiple comparisons test reveal no significant differences between the higher and lower hormone phases of OCP users ($P = 0.121$) nor the higher and lower hormone phases of non-OCP users ($P = 0.227$).

3.5 | Central arterial stiffness

There were no significant effects of group ($P = 0.499$), phase ($P = 0.682$) or interaction ($P = 0.364$) on measures of central arterial stiffness as assessed via cfPWV, as shown in Figure 2a.

3.6 | Local arterial stiffness

There were no significant effects of group (β-stiffness: $P = 0.253$, E_p: $P = 0.177$), phase (β-stiffness: $P = 0.893$, E_p: $P = 0.815$), or interaction (β-stiffness: $P = 0.190$, E_p: $P = 0.092$) on measures of β-stiffness or E_p (Figure 2).

4 | DISCUSSION

A novel finding from this study is that young healthy non-OCP women have similar central and peripheral blood pressures between the lower (EF) and higher (EL) hormone phases of a single menstrual cycle, whereas their OCP counterparts experience augmented blood

TABLE 3 Results of two-way ANOVA tests on measures of brachial systolic blood pressure (bSBP), brachial diastolic blood pressure (bDBP), brachial mean arterial pressure (bMAP), aortic systolic blood pressure (aSBP), aortic diastolic blood pressure (aDBP), aortic mean arterial pressure (aMAP), augmentation index (AIx), augmentation index standardized to a heart rate of 75 beats per minute (AIx_{75}), forward traveling wave (P_f) and backward traveling wave (P_b) during the lower hormone phases (PP, $n = 12$ and EF, $n = 10$) and higher hormone phases (AP, $n = 12$ and EL, $n = 10$) of OCP and non-OCP participants

	OCP		Non-OCP		Effect of group	Effect of phase	Group × Phase
	PP	AP	EF	EL			
bSBP (mmHg)	119.3 (8.3)	116.9 (8.7)	110.2 (8.3)	112.3 (5.0)	0.043*	0.877	0.036*
bDBP (mmHg)	70.9 (5.1)	66.5 (4.3)	68.1 (5.6)	67.6 (4.8)	0.639	0.041*	0.096
bMAP (mmHg)	87.2 (4.6)	83.3 (5.3)	82.2 (5.9)	82.5 (4.4)	0.157	0.069	0.036*
aSBP (mmHg)	104.1 (7.4)	100.1 (6.9)	96.8 (6.4)	98.2 (3.7)	0.089	0.170	0.008*
aDBP (mmHg)	73.3 (5.9)	67.0 (4.1)	68.6 (5.6)	68.6 (4.5)	0.442	0.005*	0.005*
aMAP (mmHg)	84.3 (6.9)	79.2 (4.7)	80.0 (5.3)	80.6 (4.3)	0.493	0.037*	0.010*
AIx (%)	2.4 (10.9)	2.1 (9.3)	6.2 (8.5)	5.6 (8.8)	0.355	0.713	0.916
AIx_{75} (%)	−6.7 (12.8)	−6.9 (9.3)	1.5 (9.0)	1.1 (10.4)	0.068	0.825	0.959
P_f (mmHg)	26.5 (3.8)	28.2 (5.0)	24.2 (3.0)	25.7 (2.4)	0.125	0.020*	0.896
P_b (mmHg)	12.5 (2.9)	12.3 (3.3)	10.7 (1.1)	11.3 (1.2)	0.178	0.606	0.218

Data are means (SD). *$P < 0.05$. AP, active pill; EF, early follicular; EL, early luteal; OCP, oral contraceptive pill; PP, placebo pill.

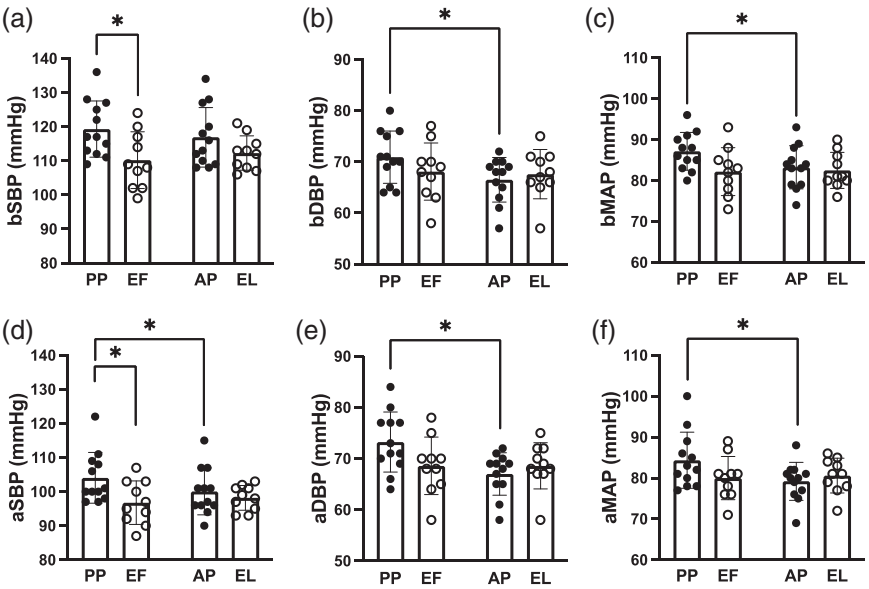

FIGURE 1 Measures of brachial systolic blood pressure (bSBP) (a), brachial diastolic blood pressure (bDBP) (b), brachial mean arterial pressure (bMAP) (c), aortic systolic blood pressure (aSBP) (d), aortic diastolic blood pressure (aDBP) (e), and aortic mean arterial pressure (aMAP) (f) during the lower hormone phases (PP, $n = 12$ and EF, $n = 10$) and higher hormone phases (AP, $n = 12$ and EL, $n = 10$) of OCP and non-OCP participants. Filled circles, OCP; open circles, non-OCP. OCP: placebo pill (PP) and active pill (AP); non-OCP: early follicular (EF) and early luteal (EL)

pressures (aSBP, bDBP, aDBP, bMAP and aMAP) in the lower hormone phase (PP) as compared to the higher hormone phase (AP). Further, results from the present study provide evidence to suggest that during the lower hormone phases (PP vs. EF), OCP users have higher central and peripheral SBP and peripheral MAP as compared to non-OCP users.

4.1 | Blood pressure changes

Significantly higher brachial SBP in OCP users during the PP phase, as compared to non-OCP users during the EF phase, as presented in the current study, is consistent with some (Harvey et al., 2015) but not all (Adler et al., 2018; Yu et al., 2014), of the existing literature. While the mechanisms leading to the increase in BP among OCP users, in general, are still incompletely understood, evidence suggests that exogenous hormones found in the combined OCP (ethinyl-oestradiol and progestin) may play a role (Enea et al., 2021). Compared to endogenous oestrogen, ethinyl-oestradiol, particularly when administered in high concentrations, exerts a stronger effect on elevating levels of coagulation factors and activation markers (Kluft & Lansink, 1997; Middeldorp et al., 2000). Elevated coagulation factors can theoretically produce prothrombotic environments that favour increases in BP. However, in combination-OCPs, various types of progestins may have

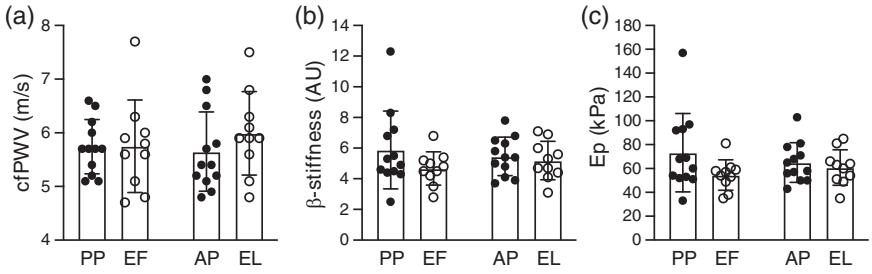

FIGURE 2 Measures of carotid-to-femoral pulse wave velocity (cfPWV) (a), β-stiffness (b), and elastic modulus (E_p) (c) during the lower hormone phases (PP, $n = 12$ and EF, $n = 10$) and higher hormone phases (AP, $n = 12$ and EL, $n = 10$) of OCP and non-OCP participants. Filled circles, OCP; open circles, non-OCP. OCP: placebo pill (PP) and active pill (AP); non-OCP: early follicular (EF) and early luteal (EL)

the ability to counteract and/or modulate the effects of ethinyl-oestradiol on coagulation (Middeldorp et al., 2000; Raps et al., 2013).

Interestingly, in the present study, the significantly higher brachial and aortic SBPs observed among OCP as compared to non-OCP users occurred only when OCP participants were withdrawn from active exogenous hormones (PP phase). This dichotomy may suggest that even in the presence of higher concentrations of ethinyl-oestradiol (as in the AP phase of OCPs), OCP use in young healthy women does not induce significantly higher BPs when compared to those of the higher hormone (EL) phase of young healthy non-OCP women. On the other hand, a potential explanation for the higher BPs among OCP users in the lower hormone phase could be supported by the hypothesis that exogenous hormones in OCPs may linger into the PP phase in those using hormonal contraception (Haaland et al., 2017; Wenner & Stachenfeld, 2020). Contrarily, the increase in ethinyl-oestradiol exposure occurring in the AP phase of OCP users does not seem to have a prolonging increased effect on BP. Taken together, these data may suggest that the relatively quicker clearing rate of endogenous oestrogen could suppress phasic variation in BP as compared to the potentially idled effects of ethinyl-oestradiol across phases.

4.2 | Arterial stiffness

While cfPWV is a reliable index of stiffening occurring at the aorta, the measure is inherently reflective of the stiffness across several arterial segments, having diverse elastic properties (Mancia et al., 2013; The Reference Values for Arterial Stiffness' Collaboration, 2010; Uejima et al., 2020). Despite reported differences in central and peripheral BPs between OCP and non-OCP users during the lower hormone phases, the data from the current study support a more consistent finding in the literature that central arterial stiffness is not affected by OCP use (Enea et al., 2021; Priest et al., 2018; Seeland et al., 2020; Yu et al., 2014) or by pill or menstrual cycle phase (Adkisson et al., 2010; Ounis-Skali et al., 2006; Priest et al., 2018; Williams et al., 2001). Results from the present study are also consistent with findings from a study by Priest et al. (2018) in terms of both central (cfPWV) and carotid arterial (β-stiffness) stiffness between OCP and non-OCP users. A noteworthy difference between the current study and Priest et al.'s study is the timing in which the non-OCP women were evaluated; while we were interested in the effects of rapidly rising levels of progesterone in the presence of increasing levels of oestrogen of non-OCP users (EL phase), Priest et al. studied women during the mid-luteal phase when it is more challenging to control for peak levels of progesterone (Priest et al., 2018). Further, the present study consisted of a cohort of OCP women using various formulations of OCPs (Table 2), whereas, in the study by Priest et al., OCP users were only using monophasic formulations (Priest et al., 2018). Taken together, these findings may suggest that various types of combined OCP formulations (i.e., monophasic, biphasic and triphasic) have similarly null effects on central and local carotid arterial stiffness. Our data, however, contrast with those of Hickson et al. (2011), who report increased measures of central arterial stiffness in OCP as compared to their non-OCP counterparts. A potential explanation for the contrasting findings could be that they did not control for menstrual cycle phase, nor report when (i.e., placebo or active pill phase) vascular measures were acquired in OCP users (Hickson et al., 2011).

4.3 | Indices of pressure waveforms

Our finding of similar measures of AIx between OCP and non-OCP users is consistent with some (Hickson et al., 2011) but not all (Enea et al., 2021; Seeland et al., 2020) of the existing literature. Contrary to our results, Robb et al. reported differences in AIx across the menstrual cycle of non-OCP users (Robb et al., 2009); however, these differences were noted between the late follicular and mid-luteal phases, which do not account for the EL surge in progesterone. Robb et al. also report that there were no significant correlations between AIx and concentrations of oestrogen or progesterone (Robb et al., 2009). Therefore, it is possible that oestrogen and progesterone may not *directly* affect the AIx within an arterial system even though the hormones have a profound effect on the vasodilatory capacity of an artery. Interestingly, OCP participants have displayed higher AIx as compared to their non-OCP counterparts suggesting that concentrations of endogenous oestrogen *are* negatively correlated with measures of AIx (Seeland et al., 2020). However, two important factors need to be considered while comparing the results of Seeland et al.'s study with the present study: (1) over ~10% of premenopausal OCP participants in the former investigation had identified as 'current smokers' in comparison to their non-OCP counterparts and (2) participants with current/past CVDs were not excluded from the analyses (Seeland et al., 2020). In contrast, in the present study, the cohort of premenopausal women were all healthy non-smokers, thus potentially explaining the differences between measures of AIx between Seeland et al.'s findings and those of the present study.

4.4 | Experimental considerations

Ideally, we would have liked to study women at various points in the menstrual and pill cycles to assess the effects of fluctuating hormones on the variables assessed in this study. However, feasibly, we were only equipped to measure women twice in one cycle. Additionally, in this study, we classified OCP use as consisting of a large range of pharmacological characteristics but narrowed our participant inclusion to include low-dose, 28-day cycle pills only. While the present study was not sufficiently powered to evaluate whether there are varying effects of different types of OCPs on haemodynamics, measures of arterial stiffness, or indices of the pressure waveform, we recognize that there are important differences among various OCP formulations and the diverse pharmacokinetic effects they may have among individuals (Goldzieher & Stanczyk, 2008). Additionally, we did not assess how differences in the duration of OCP use may or may not influence haemodynamics, parameters of arterial stiffness, or indices of the pressure waveform. However, in a recent study by Priest and colleagues (2018), no significant associations were observed between duration of OCP use and β-stiffness, or between duration of OCP use and cfPWV.

5 | CONCLUSION

Blood pressure, arterial stiffness and indices of the pressure waveform are influenced similarly with exogenous hormones (OCP users) as compared to endogenous hormones (non-OCP users). However, lower levels of exogenous hormones increase central and peripheral blood pressures.

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COMPETING INTERESTS

There are no competing interests.

AUTHOR CONTRIBUTIONS

S.M.R., L.E.E. and C.A.C. designed the research; L.E.E., C.A.C. and S.E.M. collected the data; L.E.E. analysed the data and prepared the figures; L.E.E., S.E.M. and S.M.R. interpreted the results; L.E.E. and S.M.R. drafted the manuscript; all authors edited and revised the manuscript, approved the final version and agree to be held accountable for all aspects of the work and in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, with all those qualifying for authorship being listed.

DATA AVAILABILITY STATEMENT

The data supporting findings from this study are available from upon reasonable request from the corresponding author.

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