Contraception 99 (2019) 329–334

Contents lists available at ScienceDirect

Contraception

journal homepage: www.elsevier.com/locate/con

Original article

Bone turnover markers in women participating in a dose-finding trial of a contraceptive vaginal ring releasing Nestorone and estradiol☆,☆☆,★

Molly Tiedeken [a], Carolyn L. Westhoff [a,*], Adi Cohen [b], Serge Cremers [c], Regine Sitruk-Ware [d], Diana L. Blithe [e], The NICHD Contraceptive Trials Network Vaginal Ring Group [1]

[a] Department of Obstetrics and Gynecology, and Department of Epidemiology, Columbia University Irving Medical Center, New York, NY
[b] Department of Medicine, Columbia University Irving Medical Center, New York, NY
[c] Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, NY
[d] Population Council, Center for Biomedical Research, NY, New York
[e] Contraceptive Development Program, NICHD, NIH, Bethesda, MD

ARTICLE INFO

Article history:
Received 9 August 2018
Received in revised form 1 February 2019
Accepted 18 February 2019

Keywords:
Nestorone
Segesterone
Estradiol
Contraceptive vaginal ring
Bone turnover markers

ABSTRACT

Objective: To evaluate changes in the bone turnover markers CTx and P1NP during 6 months' use of novel continuous contraceptive vaginal rings delivering Nestorone (NES) 200 mcg/day and three doses of estradiol (E2) (10, 20, and 40 mcg/day).

Study design: This randomized trial enrolled 189 women who used two consecutive vaginal rings over 180 days. Frequent blood sampling permitted analysis of NES, E2, CTx and P1NP concentrations. The bone-turnover marker analyses included only women with complete sampling and excluded women with characteristics that might interfere with accurate measurement of bone markers such as afternoon sampling, poor ring compliance or recent pregnancy. We evaluated the change from baseline to 6 months in CTx and P1NP, stratified by ring dose and by average circulating E2 concentrations.

Results: One hundred fifty-one women completed the study, and 82 women had complete data available for the bone marker analyses; the three dosage groups were balanced with regard to baseline characteristics. E2 concentrations remained low throughout treatment, regardless of which dose ring the participant used. Individual CTx changes from baseline averaged 27±56% ($p<.01$). Similarly, individual P1NP changes averaged 11±33% ($p=.04$). These increases were within the premenopausal reference ranges, and unrelated to treatment dose or to circulating E2 concentrations.

Conclusions: The low E2 dose of these rings was associated with low E2 concentrations and modest increases in serum bone turnover makers. Because we have only 6-month bone turnover markers and no direct evidence of bone loss or bone density change, these results must be interpreted with caution.

Implications: Nestorone, a 19-norprogesterone derivative, leads to complete ovarian suppression, which should yield excellent contraceptive effectiveness. To prevent potential adverse effects on bone, the NES contraceptive ring should be combined with higher doses of E2 than were assessed in this study.

© 2019 Published by Elsevier Inc.

☆ Financial Support: Support for this research was from NICHD Contraception Clinical Trial: Network HHSN275200403378I (OHSU); HHSN 275201300019I (EVMS); HHSN 275201300020I (UPenn); HHSN27521100043U (UPitt); HHSN275201300002I (NYU); HHSN275201300004I (JHU); HHSN275201300010I (Columbia). The authors also acknowledge grant support from the National Institutes of Health for the OHSU Oregon Clinical & Translational Research Institute (NIH NCRR 1 UL1 RR024120), the Bioanalytical Shared Resource/ Pharmacokinetics Core at OHSU, the Biomarkers Core Laboratory of Columbia University's Irving Institute for Clinical and Translational Research (NIH UL1 TR000040), and the work conducted at the Population Council. The content of this publication is the sole responsibility of the authors and does not necessarily represent the official views of the NIH.

☆☆ Disclosures:MT reports no conflicts of interestCW is a consultant to Merck, Bayer, Cooper Surgical, Agile Therapeutics and Allergan. Research support from Estetra SPRL, Leon Farma and Medicines360, all managed through Columbia University.

★ AC reports no conflicts of interest.SC reports no conflicts of interest.RSW is an employee of the Population Council, a not-for-profit organization, IND holder for Nestorone formulations, and developer of the vaginal ring described in this paper.DB is an employee of the NIH and is a principal investigator on a Cooperative Research and Development Agreement with HRA Pharma.

* Corresponding author. Tel.: +1 212 305 7070.
E-mail address: clw3@columbia.edu (C.L. Westhoff).

[1] The Contraceptive Clinical Trials Vaginal Ring Group members for this study include: JT Jensen, BA Chen, DF Archer, KT Barnhart, MA Thomas, AE Burke, LS Wan.

https://doi.org/10.1016/j.contraception.2019.02.012
0010-7824/© 2019 Published by Elsevier Inc.

1. Introduction

Low circulating estrogen levels are associated with bone loss and can affect skeletal development, especially in young women who may not achieve peak bone mass until age 25–30 [1–3]. Effects of hormonal contraceptives on bone density, osteoporosis, and fracture risk are unclear. Older studies suggest that past estrogen-containing OC use is associated with greater bone density in postmenopausal women, while lack of past OC use is a risk factor for postmenopausal osteoporosis [4,5]. Studies of current combination oral contraceptives in healthy premenopausal women without pre-existing estrogen deficiency have examined varying estrogen doses in populations of different ages. The majority of these studies showed no effect of oral contraceptives on bone mass [6–8]. In contrast, contraceptive methods associated with estrogen deficiency, such as depot medroxyprogesterone acetate (DMPA), are associated with increased bone remodeling markers [3,9–11], and increased bone loss assessed by dual-energy X-ray absorptiometry (DXA) [12–15]. However, cross-sectional studies have demonstrated that BMD in former adult DMPA users is similar to that of never users, providing reassurance that loss of BMD associated with DMPA use is likely transient [16–18]. The impacts on bone lead to important questions about the potential skeletal effects of new contraceptive methods. While bone loss and fractures are the endpoints of clinical interest, using intermediate endpoints may be feasible, quicker to measure, and potentially reassuring with regard to the effects of contraceptive hormones on bone.

Estrogen-deficiency-related bone loss is due to an increase in bone resorption, assessable through the measurement of bone turnover markers [19,20]. Because increases in bone resorption occur along with concomitant increases in bone formation, markers associated both with resorption and formation increase in the setting of estrogen deficiency. C-telopeptide (CTx), a marker of bone resorption, and N-terminal propeptides of procollagen type 1 (P1NP), a highly sensitive marker of bone formation, are validated surrogate markers of potential bone loss related to estrogen deficiency [21–24]. Cross-sectional studies in young women have shown consistently lower average bone turnover markers among women taking combined oral contraceptives (COCs) compared to nonusers [3,25,26], but for both markers the differences are small, and values tend to overlap [26].

An investigational, 3-month vaginal ring releasing Nestorone (NES, also known as segesterone acetate) and estradiol (E2) is under study in normal-cycling premenopausal women. The objective is to develop a user-controlled, non-oral contraceptive that delivers E2 instead of ethinyl estradiol (EE2), which is most common in oral contraceptives. NES is a 19-norprogesterone derivative that is about ten times more potent than levonorgestrel in endometrial transformation, and two times more potent in ovulation inhibition [27–29]. Oral NES is rapidly metabolized and inactive, but if administered transdermally, vaginally, or via implant, complete ovarian suppression results [27].

Because transdermal E2 administration is not associated with an increase in risk of thrombosis in postmenopausal women [30], we hypothesize that transvaginal E2 administration might also avoid increased thrombotic risk. Thus, transvaginal administration of NES with E2 could yield a contraceptive without the thrombogenic potential of oral E2 or EE2.

This Phase IIa dose-finding study evaluated a contraceptive vaginal ring (CVR) releasing NES 200 mcg/day combined with a low dose of E2, either 10, 20, or 40 mcg/day. All E2 doses resulted in lower than expected E2 serum concentrations [31]. In this study, serum E2 concentrations in most women did not reach≥40 pg/mL. Ninety-five percent of participants had a mean E2 of less than 50 pg/mL, and none of the participants had a mean E2 greater than 60 pg/mL. Thus, most participants had estradiol levels in a range (40–60 pg/mL) that has been associated with bone loss in premenopausal women in the context of ovarian suppression with GnRHa for the treatment of endometriosis [32–35]. The present analysis aims to investigate the relationship between E2 dose, serum E2 concentrations and the bone turnover markers CTx and P1NP in the women who participated in the Phase 2 study of these NES/E2 low-dose vaginal contraceptive rings. We hypothesized that women receiving the lowest E2 dose, and those achieving the lowest E2 concentrations, would have increased rates of bone turnover, demonstrated by higher concentrations of CTx and P1NP.

2. Materials and methods

The *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (Bethesda, MD) and the Population Council (New York, NY) sponsored a prospective multi-center, double-blinded, randomized, Phase 2a dose-finding study. Eight NICHD Contraceptive Clinical Trials Network (CCTN) sites participated: Columbia University, Eastern Virginia Medical School, University of Pennsylvania, University of Pittsburgh, Johns Hopkins University, Oregon Health & Science University, University of Cincinnati, and New York University. Study-related activities took place between April 2012 and December 2013. All sites obtained Institutional Review Board approval, and the trial was registered with clinicaltrials.gov (NCT0158600).

Sites recruited healthy women 18–39 years old, not at risk for pregnancy. Participants were required to have a regular menstrual cycle, intact uterus and ovaries, and a BMI ≤35 kg/m^2. Exclusion criteria included recent hormonal contraceptive use, contraindications to combined hormonal contraceptives, and use of drugs known to interfere with the metabolism of sex steroids. Participants completed a control cycle before the treatment period to confirm ovulation [31]. Health Decisions (Durham, NC) provided central randomization for all study sites using a computer-generated randomization schedule with permuted blocks. Participants were randomized 1:1:1 to receive one of three investigational CVRs all releasing NES 200 mcg/d along with E2 doses of 10 μg/day, 20 μg/day, or 40 μg/day. The Population Council formulated the silicone elastomer vaginal rings using a proprietary technology. The ring size (56.4 mm outer diameter and 8.2 mm in cross-section) and color (white) did not differ by dose. The 6-month treatment period comprised using two rings consecutively, 90 days per ring, with no hormone-free interval between the two rings. Each participant inserted the ring herself during a study visit; she inserted Ring 1 between menstrual cycle days 1–5, replaced it with Ring 2 on day 90, and removed Ring 2 on day 180.

Participants provided blood samples for hormone assays twice weekly during the baseline cycle, the first 60 days of Ring 1 use, the week prior to the ring exchange, and the last 30 days of Ring 2. Blood samples were allowed to clot, spun in a refrigerated centrifuge to separate serum, and stored at -80C until analysis. All samples underwent analysis for E2 and NES.

2.1. Lab analyses

The Biomarkers Core Laboratory of Columbia University's Irving Institute for Clinical Translational Research (New York, NY, USA) served as the central lab. E2 analyses followed liquid–liquid extraction with deuterated internal standards using an ultra-performance Liquid Chromatography–tandem Mass Spectrometry (LC-MSMS) platform. The lower limit of quantification, defined as the concentrations at which the residual of the calibration line is less than 20% of the expected concentration combined with a signal to noise ratio greater than 10, was determined to be 2.5 pg/mL for E2 with intra-assay precision of 6.3% and inter-assay precision of 8.93%. The Population Council measured NES using RIA [32]. Nelson and colleagues [33] compared E2 measurements by LC-MSMS to RIA and found strong correlation (adjusted r^2=0.97). However, for E2 concentrations below 54 pg/mL there was a moderate correlation (adjusted r^2=0.68) between LC-MSMS and RIA measurements. The lowest values of E2 found in the current analysis would likely be slightly higher had we used RIA.

CTx was measured using a Serum CrossLaps enzyme-linked immunosorbent assay (ELISA) kit from Immunodiagnostic Systems (Maryland, USA). The intra-assay precision was 2.2% and the inter-assay CV% was 7.7%. P1NP was measured by using a quantitative radioimmunoassay (RIA) kit from Orion Diagnostica (Espoo, Finland) with an intra-assay precision of 5.9% and inter-assay precision of 8%.

2.2. Data analysis

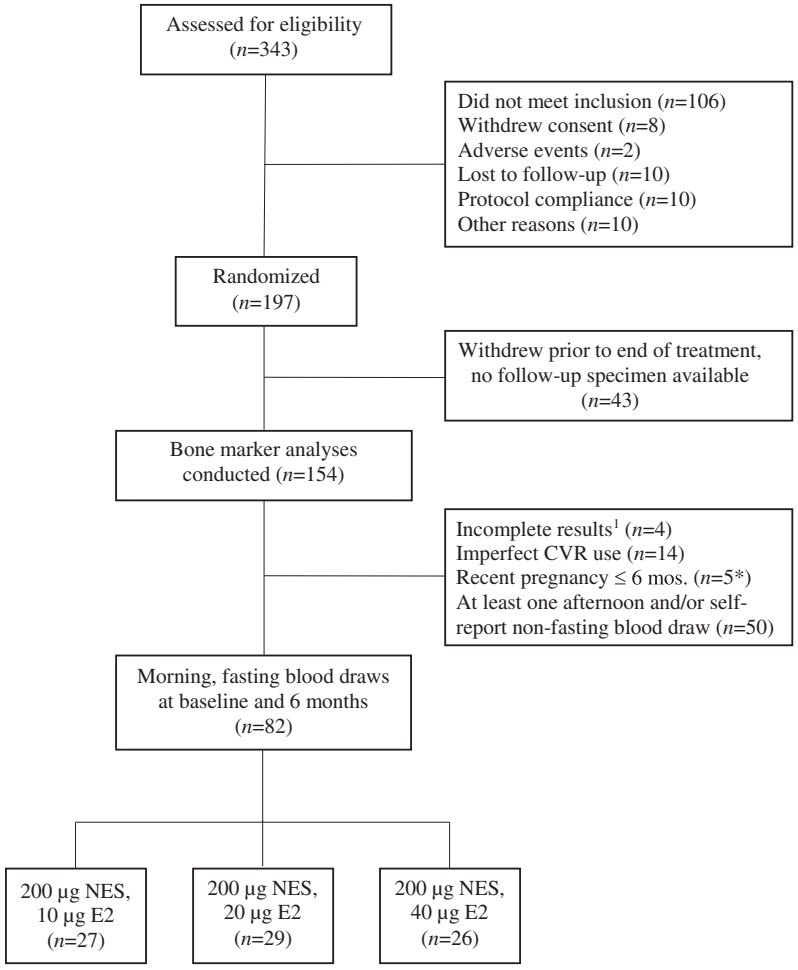
The planned sample size of approximately 189 subjects (63 subjects per group) was considered appropriate for this descriptive Phase 2a study. For the present analysis, we included treatment-compliant participants based on overall acceptable NES concentrations. Post-hoc analysis based on the observed baseline variability of CTx and P1NP indicates that we had 80% power to identify changes greater than 35% and 23%, respectively, in the 82 participants who contributed data to the bone marker analyses.

The data analysis used SAS software version 9.4 (SAS Institute, Inc. Cary, NC). We used two-way analysis of variance (ANOVA) to evaluate whether the baseline CTx and P1NP were associated with age or BMI. We then used paired sample *t* tests to evaluate the change in CTx and P1NP from baseline until after 6 months of treatment for all participants and by dose group. Because CTx exhibits strong diurnal variation, we analyzed the CTx and P1NP results only from women who contributed fasting, morning samples both at baseline and at end of treatment. We repeated the analyses using all paired samples regardless of collection time.

We assigned an average circulating E2 concentration to each participant calculated from approximately 20 samples during the treatment phase after excluding E2 results from samples taken on days of ring insertion, exchange or removal. We then categorized the mean circulating E2 concentrations into tertiles: low (<23.05 pg/mL), medium (23.05–31.21 pg/mL), and high (>31.21 pg/mL), and assessed whether E2 concentrations were related to the E2 dose of the ring. We then repeated the paired sample *t* tests to evaluate changes in CTx and P1NP within strata of low, medium, and high circulating E2 serum concentrations.

3. Results

Among the 197 participants randomized, 154 completed study procedures through the end of the treatment period (Fig. 1). Of these 154 participants, we examined the time of all blood draws and fasting status. For these analyses we retained only those participants who had morning, self-reported-fasting blood draws both at baseline and at end of treatment. The current analyses also excluded participants with



[1]Quantity not sufficient for bone marker analysis
*One participant with a recent pregnancy also had noncompliant CVR use
NES = Nestorone
E2 = Estradiol

Fig. 1. Subject enrollment and randomization in a dose-finding trial of a novel contraceptive vaginal ring containing NES and E2.

Table 1
Baseline characteristics of participants in the bone marker analysis of a dose finding trial of a novel contraceptive vaginal ring containing NES and E2

Dose NES/E2, μg/day	200/10 n=27		200/20 n=29		200/40 n=26		All subjects n=82	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	30.0	5.1	28.3	5.1	29.9	5.6	29.4	5.2
BMI (kg/m²)	26.7	4.5	25.2	4.9	24.8	3.3	25.6	4.4
Race	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Asian	2	7.4	2	6.9	2	7.7	6	7.3
Black	7	25.9	11	37.9	3	11.5	21	25.6
White	18	66.7	14	48.3	18	69.2	51	61.0
Other	0	0.0	2	6.9	3	11.5	6	6.1
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
CTx, ng/mL	0.52	0.36	0.39	0.15	0.42	0.19	0.44	0.25
P1NP, ng/mL	52.67	21.27	43.15	14.77	44.47	15.46	46.71	17.68

insufficient samples (and thus no results), participants whose NES and E2 serum concentrations indicated imperfect ring use, and those participants who had been pregnant within 6 months of study enrollment. Of the 82 remaining women, 27 received the E2 10 μg ring, 29 received the E2 20 μg ring, and 26 received the E2 40 μg ring. Women had comparable baseline characteristics across the three dose groups for age, BMI, CTx, and P1NP (Table 1). CTx and P1NP were moderately correlated with each other (r=0.51; p<.01), and both were somewhat correlated with age (r=0.31; p<.01 and r=0.25; p=.02); however, neither was correlated with BMI.

Table 2 details the mean CTx and P1NP concentrations for participants in each dose group at baseline and 6 months of continuous ring use. Individual CTx increased on average 27±56% between baseline and 6 months (p<.01); the increases seen within each dose group were compatible with the overall change. Individual P1NP increased on average 11±33% from baseline to 6 months (p=.04). The dose-group P1NP increases were compatible with the overall increase.

Table 2
CTx and P1NP at baseline and after 6 months of ring use by dose group in a dose finding trial of a novel contraceptive vaginal ring

	N	Mean	SD	95% Confidence Interval	p-value*
a. CTx					
CTx, ng/mL					
10 μg E2	27				
Baseline		0.52	0.36	(0.38–0.66)	.35
6 months		0.55	0.32	(0.42–0.68)	
20 μg E2	29				
Baseline		0.39	0.15	(0.33–0.45)	.002
6 months		0.50	0.18	(0.43–0.57)	
40 μg E2	26				
Baseline		0.42	0.19	(0.34–0.49)	.01
6 months		0.50	0.26	(0.40–0.61)	
Total	82				
Baseline		0.44	0.25	(0.39–0.50)	<.0001
6 months		0.52	0.26	(0.46–0.57)	
b. P1NP					
P1NP, ng/mL					
10 μg E2	27				
Baseline		52.67	21.27	(44.26–61.09)	.83
6 months		53.29	17.27	(46.46–60.12)	
20 μg E2	29				
Baseline		43.15	14.77	(37.53–48.77)	.02
6 months		47.98	16.21	(41.81–54.14)	
40 μg E2	26				
Baseline		44.47	15.46	(38.23–50.72)	.16
6 months		47.01	16.86	(40.20–53.82)	
Total	82				
Baseline		46.71	17.68	(42.82–50.59)	.04
6 months		49.42	16.79	(45.73–53.11)	

* Paired sample t test.

Table 3
Number of participants per dose group who had low, medium, and high average circulating E2 serum concentrations in a dose-finding trial of a novel contraceptive vaginal ring

	Randomization dose group			All participants
	10 μg E2	20 μg E2	40 μg E2	
Mean E2*	28.2 (9.8)	28.8 (12.8)	26.6 (10.3)	27.9 (11.0)
Low E2[1]	6	10	11	27
Medium E2[2]	11	8	9	28
High E2[3]	10	11	6	27

$\chi^2_4 = 3.49$, p=.48

* Mean E2 was averaged over approximately 20 samples, not including days when the ring was changed, or removed at end of treatment

[1] Low E2<23.05 pg/mL

[2] Medium E2 23.05–31.21 pg/mL

[3] High E2>31.21 pg/mL

Table 3 shows the distribution of E2 concentrations in each dose group; the E2 concentrations were unrelated to the ring dose. Table 4 shows the CTx and P1NP concentrations at 6 months in subgroups stratified by ring dose and by E2 concentrations. Finally, the Appendix table shows the baseline and 6-month CTx and P1NP concentrations according to tertiles of E2 concentration (analogous to Table 2). Mean serum CTx and P1NP concentrations increased between baseline and 6 months and within the dose and E2 concentration subgroups. When we ignored the time of the blood draws and included data from the 50 women with afternoon and/or non-fasting sampling, those analyses yielded similar results (data not shown).

4. Discussion

Initial analyses from this randomized trial [31] revealed that the median E2 serum concentrations during ring use did not achieve, in most participants, the minimum levels that may be necessary for bone protection. The mean E2 serum level to prevent bone loss in premenopausal women is suggested to be≥40 pg/mL [32,34,35], and over 80% of women in this study had average E2 concentrations below that level. Therefore, we carried out the present analyses to investigate if women using the study rings for 6 months experienced increases in the bone turnover markers CTx and P1NP. Such increases would indicate a potential for bone loss. Means for CTx and P1NP increased on average 27±56% and 11±33% respectively during investigational CVR use, but the 6-month levels of CTx and P1NP were still within the premenopausal reference ranges (CTx: 0.11–0.74 ng/mL; P1NP: 19–83 ng/mL). Individual changes were quite variable, and the magnitude of the increases was unrelated to dose group (E2, 10, 20, or 40 μg) or to average circulating E2 serum concentrations (low, medium, or high). That is, we did not observe the expected dose–response relationship between bone

Table 4
Mean CTx after using a novel CVR and 6 months stratified by dose group and by E2 serum concentrations

		Randomization dose group			All doses together
	n	10 μg E2	20 μg E2	40 μg E2	
a. CTx					
All participants	82	0.55 (0.32)	0.50 (0.18)	0. 50 (0.26)	0.52 (0.26)
Low E2[1]	27	0.75 (0.61)	0.48 (0.14)	0.46 (0.15)	0.53 (0.32)
Medium E2[2]	28	0.46 (0.14)	0.51 (0.25)	0.56 (0.23)	0.51 (0.20)
High E2[3]	27	0.52 (0.17)	0.50 (0.18)	0.51 (0.43)	0.51 (0.24)
b. P1NP					
All participants	82	53.29 (17.27)	47.98 (16.21)	47.01 (16.86)	49.42 (16.79)
Low E2[1]	27	49.87 (11.80)	50.02 (22.15)	44.68 (10.04)	47.81 (15.57)
Medium E2[2]	28	48.65 (14.26)	52.73 (10.76)	51.47 (16.51)	50.72 (13.75)
High E2[3]	27	60.44 (21.62)	42.66 (12.66)	44.58 (27.00)	49.67 (20.86)

[1] Low E2<23.05 pg/mL

[2] Medium E2 23.05–31.21 pg/mL

[3] High E2>31.21 pg/mL

markers and the ring dose group or the resultant estradiol concentrations. We are unsure why we did not observe the expected dose–response relationship; this may be due to individual factors such as drug absorption, diet, and exercise [36].

The 27% CTx increase after 6 months of investigational CVR use is substantially lower than changes in bone remodeling markers seen with natural menopause, surgical menopause, and changes in women with estrogen deficiency related to treatment with GnRH agonists. After surgical menopause, Peris and colleagues [37] documented a 200% increase in CTx after 3 months, which then declined by 50% after 3 months of transdermal estradiol hormone replacement therapy. Bahar and colleagues [38] documented a 54% increase in CTx 1 month after surgical menopause and 130% increase after 6 months. Urinary NTX, a marker of bone resorption closely related to CTx, has documented increases after natural menopause of approximately 40% over 4 years during the menopausal transition [37]. Similarly, GnRH agonist use in premenopausal women leads to an increase of approximately 40% in NTX over 3 months [39,40]. Thus, the more modest changes reported here could be viewed as reassuring.

A limitation of the current study is the lack of direct assessment of bone mass change using serial measures by DXA. Bone mineral density changes would, however, be difficult to detect within the 6-month timeframe of this study. In contrast, bone turnover marker (CTx and PINP) changes are expected to occur at this early time point and can be predictive of later bone mass changes [21–24]. Thus, we relied on bone turnover markers as harbingers for bone mass change.

NuvaRing, marketed in the US since 1990, releases EE2 15 μg/day and etonorgestrel 120 μg/day [41]. In contrast to the findings in this study, NuvaRing has been associated with a decrease in a bone resorption markers at 6 months compared to baseline, and with no change in bone density at one and 2 years [42,43]. As EE2 is a more potent estrogen than E2, those results may not be relevant to effects of the rings studied here. The FDA recently approved a second contraceptive vaginal ring, Annovera, containing segesterone acetate and ethinyl estradiol; the impact of Annovera on bone health is not yet known.

Brache et al. [44] previously tested NES-only rings delivering 50, 75 and 100 mcg/d, and found that low E2 levels (≤100 pmol/L, i.e., 30 pg/mL) occurred only with the ring delivering NES 100 mcg /day), and not with lower-dose NES-only rings. The rings in this study delivered NES 200 mcg/day, which may be desirable as complete ovarian suppression should yield outstanding contraceptive effectiveness, but using this NES dose will require more E2 delivered in the ring to compensate for the complete ovarian suppression. The E2 doses selected for this dose-finding study did not provide sufficient E2 replacement in the setting of such highly suppressed ovarian function. Based on the bone-turnover marker findings, we conclude that the E2 doses used in the CVR investigated here may not provide adequate bone protection. Due to these concerns, the NES/E2 ring prototypes have been re-engineered and current ring studies are investigating higher doses of E2 in combination with NES.

Acknowledgements

The authors would like to thank Bruce Variano at the Population Council for engineering the rings, Narendar Kumar at the Population Council for developing and carrying out the NES assays, and Clint Dart at Health Decisions for assistance with data management and analysis.

Appendix A. CTx and P1NP at baseline and after 6 months of ring use by average E2 concentrations in a dose finding trial of a novel contraceptive vaginal ring

	N	Mean	SD	95% Confidence Interval	Paired sample *t* test p-value
a. CTx					
CTx, ng/mL					
Low E2[1]	27				
Baseline		0.47	0.37	(0.33, 0.62)	0.07
6 months		0.53	0.32	(0.40, 0.66)	
Medium E2[2]	28				
Baseline		0.43	0.13	(0.38, 0.48)	0.02
6 months		0.51	0.20	(0.43, 0.59)	
High E2[3]	27				
Baseline		0.43	0.22	(0.34, 0.51)	0.01
6 months		0.51	0.24	(0.42, 0.61)	
Total	82				
Baseline		0.44	0.25	(0.39, 0.50)	<0.0001
6 months		0.52	0.26	(0.46, 0.57)	
b. P1NP					
P1NP, ng/mL					
Low E2[1]	27				
Baseline		47.09	18.58	(39.74, 54.43)	0.75
6 months		47.81	15.57	(41.65, 53.97)	
Medium E2[2]	28				
Baseline		48.41	15.33	(42.47, 54.36)	0.27
6 months		50.72	13.75	(45.39, 56.05)	
High E2[3]	27				
Baseline		44.56	19.40	(36.89, 52.23)	0.04
6 months		49.67	20.86	(41.42, 57.92)	
Total	82				
Baseline		46.71	17.68	(42.82, 50.59)	0.04
6 months		49.42	16.79	(45.73, 53.11)	

[1] Low E2 < 23.05 pg/mL
[2] Medium E2 23.05 – 31.21 pg/mL
[3] High E2 > 31.21 pg/mL

References

[1] Recker RR, Davies KM, Hinders SM, Heaney RP, Stegman MR, Kimmel DB. Bone gain in young adult women. JAMA 1992;268:2403–8.
[2] Lofman O, Larsson L, Toss G. Bone mineral density in diagnosis of osteoporosis: Reference population, definition of peak bone mass, and measured site determine prevalence. J Clin Densitom 2000;3:177–86.
[3] Ott SM, Scholes D, LaCroix AZ, Ichikawa LE, Yoshida CK, Barlow WE. Effects of contraceptive use on bone biochemical markers in young women. J Clin Endocrinol Metab 2001;86:179–85.
[4] Stevenson JC, Lees B, Devenport M, Cust MP, Ganger KF. Determinants of bone density in normal women: risk factors for future osteoporosis? BMJ 1989;298 (6678):924–8.
[5] Stevenson JC, Cust MP, Gangar KF, Hillard TC, Lees BL, Whitehead MI. Effects of transdermal versus oral hormone replacement therapy on bone density in spine and proximal femur in postmenopausal women. Lancet 1990;336(8710):265–9.
[6] Liu SL, Lebrun CM. Effect of oral contraceptives and hormone replacement therapy on bone mineral density in premenopausal and perimenopausal women: a systematic review. Br J Sports Med 2006;40(1):11–24.
[7] Wei S, Venn A, Ding C, Foley S, Laslett L, Jones G. The association between oral contraceptive use, bone mineral density and fractures in women aged 50-80 years. Contraception 2011;84(4):357–62.
[8] Lopez LM, Grimes DA, Schulz KF, Curtis KM, Chen M. Steroidal contraceptives: Effect on bone fractures in women. The Cochrane library; 2014.
[9] Naessen T, Olsson S, Gudmundson J. Differential effects on bone density of progestogen-only methods for contraception in premenopausal women. Contraception 1995;52(1):35–9.
[10] Shaarawy M, El-Mallah SY, Seoudi S, Hassan M, Mohsen IA. Effects of the long-term use of depot medroxyprogesterone acetate as hormonal contraceptive on bone mineral density and biochemical markers of bone remodeling. Contraception 2006;74:297–302.
[11] Walsh JS, Eastell R, Peel NF. Effects of depot medroxyprogesterone acetate on bone density and bone metabolism before and after peak bone mass: a case-control study. J Clin Endocrinol Metab 2008;93:1317–23.
[12] Berenson AB, Radecki Breitkopf C, Grady JJ, Rickert VI, Thomas A. Effects of hormonal contraception on bone mineral density after 24 months of use. Obstet Gynecol 2004; 103:899–906.
[13] Clark MK, Sowers M, Nichols S, Levy B. Bone mineral density changes over two years in first-time users of depot medroxyprogesterone acetate. Fertil Steril 2004;82: 1580–6.
[14] Scholes D, Lacroix AZ, Ichikawa LE, Barlow WE, Ott SM. Change in bone mineral density among adolescent women using and discontinuing depot medroxyprogesterone acetate contraception. Arch Pediatr Adolesc Med 2005;159:139–44.
[15] Kyvernitakis I, Kostev K, Nassour T, Thomasius F, Hadji P. The impact of depot medroxyprogesterone acetate on fracture risk: a case-control study from the UK. Osteoporos Int 2017;28(1):291–7.
[16] Orr-Walker BJ, Evans MC, Ames RW, Clearwater JM, Cundy T, Reid IR. The effect of past use of the injectable contraceptive depot medroxyprogesterone acetate on bone mineral density in normal post-menopausal women. Clin Endocrinol (Oxf) 1998;49:615–8.
[17] Petitti DB, Piaggio G, Mehta S, Cravioto MC, Meirik O. Steroid hormone contraception and bone mineral density: a cross-sectional study in an international population. The WHO Study of Hormonal Contraception and Bone Health. Obstet Gynecol 2000;95:736–44.
[18] Viola AS, Castro S, Marchi NM, Bahamondes MV, Viola CF, Bahamondes L. Long-term assessment of forearm bone mineral density in postmenopausal former users of depot medroxyprogesterone acetate. Contraception 2011;84(2):122–7.
[19] Seibel MJ. Biochemical markers of bone turnover part II: clinical applications in the management of osteoporosis. Clin Biochem Rev 2006;27:123–38.
[20] Delmas PD, Eastell R, Garnero P, et al. The use of biochemical markers of bone turnover in osteoporosis. Committee of Scientific Advisors of the International Osteoporosis Foundation. Osteoporos Int 2000;11(Suppl. 6):S2–17.
[21] Bauer DC, Sklarin PM, Stone KL, Black DM, Nevitt MC, Ensrud KE, et al. Biochemical markers of bone turnover and prediction of hip bone loss in older women: the study of osteoporotic fractures. J Bone Miner Res 1999;14(8):1404–10.
[22] Chaki O, Yoshikata I, Kikuchi R, Nakayama M, Uchiyama Y, Hirahara F, et al. The predictive value of biochemical markers of bone turnover for bone mineral density in postmenopausal Japanese women. J Bone Miner Res 2000;15(8):1537–44.
[23] Ivaska KK, Lenora J, Gerdhem P, Åkesson K, Väänänen HK, Obrant KJ. Serial assessment of serum bone metabolism markers identifies women with the highest rate of bone loss and osteoporosis risk. J Clin Endocrinol Metab 2008;93(7):2622–32.
[24] Vasikaran S, Eastell R, Bruyère O, Foldes AJ, Garnero P, Griesmacher A, et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. Osteoporos Int 2011;22 (2):391–420.
[25] Garnero P, Sornay-Rendu E, Delmas PD. Decreased bone turnover in oral contraceptive users. Bone 1995;16(5):499–503.
[26] de Papp AE, Bone HG, Caulfield MP, Kagan R, Buinewicz A, Chen E, et al. A cross-sectional study of bone turnover markers in healthy premenopausal women. Bone 2007;40(5):1222–30.
[27] Kumar N, Koide SS, Tsong YY, Sundaram K. Nestorone®: a progestin with a unique pharmacological profile. Steroids 2000;65(10):629–36.
[28] Sitruk-Ware R. Pharmacological profile of progestins. Maturitas 2004;47(4):277–83.
[29] Sitruk-Ware R. New progestogens for contraceptive use. Hum Reprod Update 2005; 12(2):169–78.
[30] Rovinski D, Ramos RB, Fighera TM, Casanova GK, Spritzer PM. Risk of venous thromboembolism events in postmenopausal women using oral versus non-oral hormone therapy: a systematic review and meta-analysis. Thromb Res 2018;168:83–95.
[31] Jensen JT, Edelman AB, Chen BA, Archer DF, Barnhart KT, Thomas MA, et al. Continuous dosing of a novel contraceptive vaginal ring releasing Nestorone® and estradiol: pharmacokinetics from a dose-finding study. Contraception 2018;97(5):422–7.
[32] Reginster JY, Sarlet N, Deroisy R, Albert A, Gaspard U, Franchimont P. Minimal levels of serum estradiol prevent postmenopausal bone loss. Calcif Tissue Int 1992;51(5):340–3.
[33] Nelson RE, Grebe SK, O'Kane DJ, Singh RJ. Liquid chromatography–tandem mass spectrometry assay for simultaneous measurement of estradiol and estrone in human plasma. Clin Chem 2004;50(2):373–84.
[34] Hornstein MD, Surrey ES, Weisberg GW, Casino LA. Lupron add-Back study group. Leuprolide acetate depot and hormonal add-back in endometriosis: a 12-month study. Obstet Gynecol 1998;91(1):16–24.
[35] Finkelstein JS, Klibanski A, Arnold AL, Toth TL, Hornstein MD, Neer RM. Prevention of estrogen deficiency–related bone loss with human parathyroid hormone–(1-34): a randomized controlled trial. JAMA 1998;280(12):1067–73.
[36] Delmas PD, Eastell R, Garnero P, Seibel MJ, Stepan J. The use of biochemical markers of bone turnover in osteoporosis. Osteoporosis Int 2000;11(18):S2–17.
[37] Peris P, Alvarez L, Monegal A, Guanabens N, Duran M, Pons F, et al. Biochemical markers of bone turnover after surgical menopause and hormone replacement therapy. Bone 1999;25(3):349–53.
[38] Bahar S, Abali R, Guzel S, Bozkurt S, Guzel EC, Aral H, et al. Comparison of the acute alterations in serum bone turnover markers and bone mineral density among women with surgical menopause. Eur J Obstet Gynecol Reprod Biol 2011; 159(1):194–7.
[39] Sowers MR, Zheng H, Greendale GA, Neer RM, Cauley JA, Ellis J, et al. Changes in bone resorption across the menopause transition: effects of reproductive hormones, body size, and ethnicity. J Clin Endocrinol Metab 2013;98(7):2854–63.
[40] Kukuvitis A, Bildiris J, Prapas Y, Tsamis D, Papadimas J, Dimitriadou A, et al. Effect of treatment with GnRH agonists on new markers of bone metabolism. Gynecol Obstet Invest 1999;47(3):194–6.
[41] Wieder DR, Pattimakiel L. Examining the efficacy, safety, and patient acceptability of the combined contraceptive vaginal ring (NuvaRing®). Int J Womens Health 2010;2:401.
[42] Massai R, Mäkäräinen L, Kuukankorpi A, Klipping C, Duijkers I, Dieben T. The combined contraceptive vaginal ring and bone mineral density in healthy premenopausal women. Hum Reprod 2005;20(10):2764–8.
[43] Massaro M, Di Carlo C, Gargano V, Formisano C, Bifulco G, Nappi C. Effects of the contraceptive patch and the vaginal ring on bone metabolism and bone mineral density: a prospective, controlled, randomized study. Contraception 2010;81(3):209–14.
[44] Brache V, Mishell DR, Lahteenmaki P, Alvarez F, Elomaa K, Jackanicz T, et al. Ovarian function during use of vaginal rings delivering three different doses of Nestorone. Contraception 2001;63:257–61.