Long-Term Use of Contraceptive Depot Medroxyprogesterone Acetate in Young Women Impairs Arterial Endothelial Function Assessed by Cardiovascular Magnetic Resonance

Morten B. Sorensen, PhD; Peter Collins, MD; Paul J.L. Ong, MA; Carolyn M. Webb, PhD; Christopher S. Hayward, MD; Elizabeth A. Asbury, MSc; Peter D. Gatehouse, PhD; Andrew G. Elkington, MB, BS; Guang Z. Yang, PhD; Ali Kubba, MB, ChB; Dudley J. Pennell, MD

Background—Depot medroxyprogesterone acetate (DMPA) inhibits proliferation of ovarian follicles, resulting in anovulation and a decrease in circulating estrogen; the latter action is potentially disadvantageous to cardiovascular health. We therefore investigated the vascular effects of long-term contraceptive DMPA in young women.

Methods and Results—Endothelium-dependent (hyperemia-induced flow-mediated dilatation [FMD]) and -independent (glyceryl trinitrate [GTN]) changes in brachial artery area were measured using cardiovascular magnetic resonance in 13 amenorrheic DMPA users (>1 year use; mean age 29±4 years) and in 10 controls (mean age 30±4 years, $P=0.25$) with regular menstrual cycles after validation of the technique. FMD and GTN responses were measured just before repeat MPA injection and 48 hours later (n=12) in DMPA users and during menstruation and midcycle (n=9) in controls. Serum-estradiol levels (S-estradiol) were measured at both visits. FMD was reduced in DMPA users compared with controls during menstruation (1.1% versus 8.0%, respectively $P<0.01$) without differences in GTN responses. S-estradiol levels in DMPA users were significantly lower than in controls during menstruation (58 versus 96 pmol/L, $P<0.01$). High levels of circulating MPA 48 hours after injection were not linked to an additional impairment in FMD (2.0% versus 3.1%, $P=0.23$). Estradiol levels were significantly correlated to FMD ($r=0.43$, $P<0.01$).

Conclusions—Endothelium-dependent arterial function measured by cardiovascular magnetic resonance is impaired in chronic users of DMPA, and hypoestrogenism may be the mechanism of action. DMPA might adversely affect cardiovascular health, and in particular its use in women with cardiovascular disease should be additionally evaluated. (***Circulation***. 2002;106:1646-1651.)

Key Words: endothelium ■ magnetic resonance imaging ■ women ■ heart disease

Depot medroxyprogesterone acetate (DMPA) is a widely used long-acting contraceptive, given as a 150 mg IM injection every 12 weeks. Contraception is achieved mainly via interference with the hypothalamic-pituitary-ovarian axis with inhibition of gonadotropin release at the pituitary level and subsequent anovulation.[1,2] The induced follicular stagnation is associated with decreased circulating estrogen,[1] which has been linked to adverse effects on estrogen-sensitive tissues.[3,4] DMPA-induced hypoestrogenism might also affect vascular function. Estrogens have powerful vasoactive properties that inhibit atheroma formation partly via endothelial effects.[5] This may explain why oophorectomy and premature ovarian failure have been linked to accelerated cardiovascular disease. In addition to the possible adverse vascular effects of follicular arrest, MPA may have unwanted effects on arterial function. It has been suggested that MPA coadministration with estrogen for hormone replacement is linked to atheroma development,[6] which might be attributed to adverse effects on the endothelium.[7] In view of these data and the fact that DMPA is used for contraception in premenopausal women with cardiovascular disease,[8] we assessed the effects of long-term DMPA use on endothelial function.

Endothelium-dependent arterial relaxation[9] is attributable to endothelium-derived NO.[10] In arteries lined by healthy

TABLE 1. Characteristics of 13 Amenorrheic Users of Contraceptive Progestogen Injection (DMPA) and 10 Controls With Regular Menstruation

	DMPA Users	Controls
Age, y	29.0±4.1	30.2±4.2
Body mass index, kg · m⁻²	23.5±3.7	25.0±4.0
Systolic blood pressure, mm Hg	108±11	108±13
Diastolic blood pressure, mm Hg	70±7	70±8
Total cholesterol, mmol/L	4.2±0.8*	5.0±0.7
HDL cholesterol, mmol/L	1.4±0.2*	1.8±0.2
Menopausal symptoms	0.92±1.6*	0
Length of DMPA use, mo	52±46*	0
Length of amenorhea, mo	51±47*	0

Values are mean±SD.

*$P < 0.05$ vs controls.

endothelium, blood flow increase causes NO release and arterial dilatation. The degree of arterial dilatation in response to an increase in blood flow is used as an index of endothelial functional integrity. Endothelial dysfunction is associated with early atheroma formation and is linked to an increase in future cardiac events.[11,12] Celermajer et al[13] described the use of external ultrasound imaging (EXUS) to measure flow-mediated dilatation (FMD) and nitrate-induced dilatation of the brachial artery (BA) (together brachial artery reactivity [BAR]) as a marker of vascular endothelial and nonendothelial function, respectively. However, reports have questioned the accuracy and sensitivity of the method.[14,15] Cardiovascular magnetic resonance (CMR) is increasingly being used to assess cardiovascular function, and recent improvements in spatial resolution and tissue differentiation make CMR very reproducible.[16] When using CMR, two-dimensional imaging of the arterial cross section is achieved compared with one-dimensional imaging with EXUS.

The aim of the present study was to investigate, in premenopausal women without known risk of endothelial dysfunction, the effect of chronic DMPA use on endothelium-dependent and -independent arterial function measured by CMR.

Methods

Participants

Thirteen long-term DMPA users (mean age 29±4 years) and 10 controls (mean age 30±4 years) with a regular menstrual cycle and no intake of progestogens were enrolled. Only DMPA users for >1 year with long-lasting (>1 year) secondary amenorrhea were included. It was ensured that DMPA users had regular menstrual cycles before commencing DMPA, were not under ideal body weight (BMI >19), and did not have a history of eating disorder or excessive exercise performance. Neither DMPA users nor controls had been prescribed estrogens or other vasoactive medication up to 3 months before inclusion, and they were excluded if any of the following factors known to cause endothelial dysfunction were present: coronary artery disease (CAD), diabetes, habitual cigarette smoking, hypertension, dyslipidemia, obesity (BMI >31), or a family history of CAD in a first-degree relative <55 years of age. Total cholesterol and HDL cholesterol levels were measured at inclusion in all subjects, and normal values were a requirement for inclusion. Patient characteristics are shown in Table 1.

Study Design

Arterial function was assessed twice by CMR. In the DMPA users, CMR was timed in accordance with the injection regimen (just before repeat injection and 48 hours later at highest circulating levels of MPA).[2] In controls, CMR was timed in accordance with the menstrual cycle. The first study was performed during the menstrual phase (cycle days 1 through 3, at the expected time of lowest circulating estradiol levels), and the second study was performed midcycle (two days before to day of ovulation, as calculated from expected day of subsequent period minus 14 days, at the expected time of highest circulating estradiol levels). CMR was performed after a 6-hour fast, at the same time of the day, and after at least a half-hour sitting rest. Before the first study, all participants were asked if they experienced any of the following complaints, which have been linked to estrogen deficiency: flushing, night sweats, sleeplessness, abdominal weight gain, and vaginal dryness/atrophy. Estradiol levels were measured at both visits by radioimmunoassay. The study was approved by the local ethics committee, and all subjects gave written informed consent.

Validation and Reproducibility Studies

To validate the CMR technique, we studied 8 male patients referred for diagnostic coronary angiography (mean age 58±8 years). These subjects underwent measurement of BAR by CMR, intravascular ultrasound (IVUS), and external ultrasound (EXUS). The EXUS and
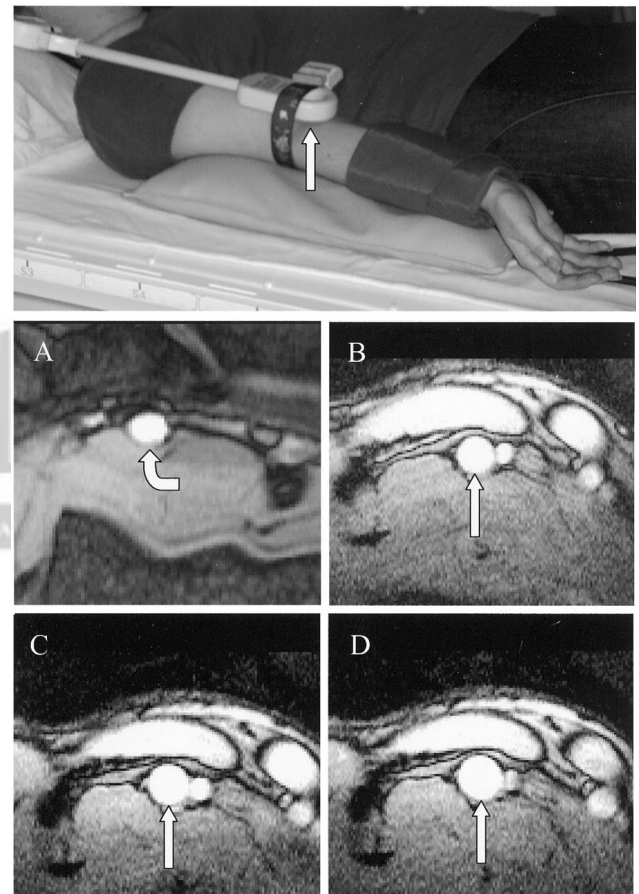
Figure 1. The upper image shows the set up for CMR. The surface coil (arrow) and the cuff are mounted. Movement is inhibited by sand bags. A, Baseline transaxial image of the brachial artery (curved arrow) in a healthy subject. The artery appears oval. B through D, Series of images in a healthy subject. B, Baseline image; C, During peripheral reactive hyperaemia; and D, After a sublingual dosage of GTN. Note the significant increase in area of the brachial artery (arrows).

TABLE 2.   Comparison of Test-Retest Repeatability of CMR and EXUS for Measurement of Arterial Cross-Sectional Diameter/Area, FMD, and GTN Response

	Mean±SD	Mean Difference±SD of 2 Measurements	Coefficient of Variability
Baseline parameter			
CMR area, mm^2	14.9±3.9	−0.45±1.6	0.11
EXUS diameter, mm	4.5±0.66	−0.02±0.44	0.10
EXUS area, mm^2	15.9±4.8	−0.17±3.0	0.19*
Flow-mediated dilatation			
CMR area, %	8.4±8.0	0.6±2.0	0.23
EXUS diameter, %	7.1±8.2	1.7±5.8	0.81*
EXUS area, %	15±18	3.6±12	0.80*
Response to glyceryl trinitrate			
CMR area, %	33.5±7.0	5.9±7.1	0.21
EXUS diameter, %	14±6.9	1.6±7.8	0.54
EXUS area, %	31±16	3.2±18	0.57

The coefficient of variability by CMR is significantly lower than by EXUS.

*$P<0.05$, compared with CMR area.

IVUS measurements were performed simultaneously after diagnostic coronary angiography, and CMR was performed a few days later. The subjects were examined fasting and were asked to withhold vasoactive medication for 24 hours before examinations. CAD was defined as at least one significant coronary artery stenosis with >70% reduction in calibre, as assessed by an independent cardiologist. To compare BAR measurements by CMR and EXUS, in 11 healthy males (mean age 32±3 years), we performed one study by each of the two methods on separate days, less than 1 week apart. To compare repeatability of BAR by CMR and EXUS, we performed repeated measurements in 7 subjects, who had a total of 4 measurements of FMD and GTN responses.

Measurement of Arterial Reactivity

BAR was measured using 3 different imaging techniques: CMR, IVUS, and EXUS. CMR measurement of BA area was performed at baseline and 1 minute after reactive hyperemia was induced by release of a forearm cuff inflated to suprasystolic pressure for 5 minutes. Measurement of non–endothelium-dependent vascular reactivity was performed by imaging and 3 minutes after 400 μg sublingual GTN. The BA was imaged with high-resolution CMR using the following parameters: a segmented FLASH gradient echo sequence; 8 views per segment; TE 14 ms; field of view 7×3.5 cm; matrix size 256×128, pixel size 0.27×0.27 mm, and acquisition time 12 cardiac cycles; and diastolic trigger delay. Imaging was performed with a 1.5T Picker Edge scanner with a small loop surface coil attached to the right elbow (left elbow in validation studies). The imaging position was reproduced by using the transaxial plane where the BA was most superficial, and perpendicular position was assured by 3-dimensional piloting (Figure 1). BA area was measured objectively by tracking of the arterial region of interest by in-house developed autosegmentation software. The BA area at the intimal border was delineated automatically in triplicate and the area was averaged (CMRtools, Imperial College). Because each scan was identifiable via a unique number, batch analysis was performed blinded in random order after completing the studies.

Measurement of arterial function by IVUS was performed after diagnostic coronary angiography. A 3F 20-MHz IVUS probe (Endosonics Visions-Five-64) was positioned in the left BA. Measurement of BA area was made at baseline and 1 minute after reactive hyperaemia (induced by release of a forearm cuff inflated to suprasystolic pressure for 5 minutes). Non–endothelium-dependent BA responses were then measured 3 minutes after 300 μg intrabrachial isosorbide dinitrate. Measurements were performed in triplicate using planimetry by an independent investigator.

Measurement of arterial function by EXUS was performed by a standard technique, as previously described.[17] The study group is experienced with the EXUS technique.[17,18] Two EXUS observers performed the data collection; one (P.O.) collected data for the validation studies, and another (E.A.) collected data for the reproducibility and repeatability comparisons in the healthy volunteers. To compare EXUS and CMR directly, the derived area from EXUS diameter (d) was also calculated by $\pi(d/2)^2$.

Statistical Analysis

A paired/unpaired *t* test was used for intrasubject and group comparisons of effects. Pearson's correlation coefficient was used for assessment of association after logarithmic transformation of estradiol levels. The coefficient of variability (CV) was calculated as the SD of the difference of repeated measures divided by the mean measurement value. The repeatability of the 2 methods was com-
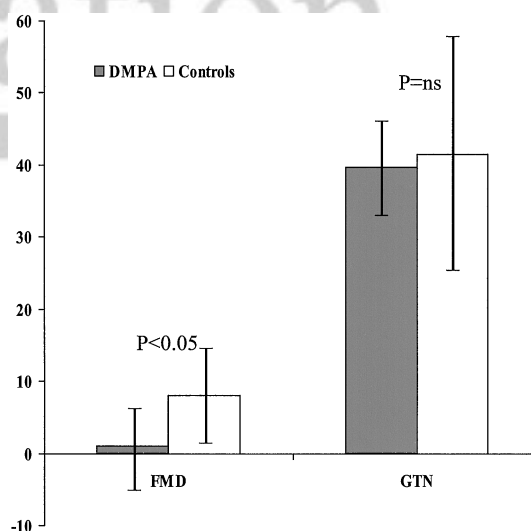

Figure 2. Differences in endothelium-dependent (FMD) and endothelium-independent (GTN) arterial reactivity in long-term users of DMPA and healthy controls. FMD and GTN are both assessed by measurement of area changes by cardiovascular magnetic resonance. FMD in the DMPA patients is significantly impaired compared with controls, whereas the GTN responses are equivalent.

TABLE 3.   Repeated Measurements of FMD and GTN Response by CMR Area and S-oestradiol Levels in DMPA Users and Controls

	FMD, %	GTN Response, %	S-oestradiol, pmol/L
Controls (n=9)			
Menstruation	7.2±4.4	40.2±18.2	91.6±28.9
Ovulation	12.5±6.5*	39.6±21.2	682±476*
DMPA users (n=12)			
Baseline	2.0±4.9†	42.0±10.8	64.6±38.5†
48 Hours after injection	3.1±5.7†	47.5±12.0	55.1±29.5†

Values are mean±SD.

Menstruation: First measurement was performed at cycle day 1 to 4. Ovulation: Second visit was performed from <2 days before the calculated day of ovulation, on the basis of average cycle length.

*$P<0.05$ vs previous measurement.

†$P<0.05$ vs controls.

pared by performing a 2-tailed paired t test on the logged squared test-retest differences (Bland JM. Comparing within-subject variances in a study to compare two methods of measurement. Available at: http://www.sghms.ac.uk/depts/phs/staff/jmb/compsd.htm. Accessed July 25, 2002). Data are presented as mean±SD, and $P<0.05$ was considered significant.

We calculated the required sample sizes to detect significant differences in FMD by CMR based on our reproducibility data (Table 2). It has previously been shown that hypoestrogenism in premenopausal women treated with a GnRH agonist is linked to a 10% reduced FMD measured by EXUS.[19] A sample size of 20 subjects (10 subjects in each group) would be required to detect by CMR a 3% difference in FMD between DMPA and control groups with $\alpha=0.05$ and a power of 90%.

Results

Validation of CMR for Assessment of Arterial Reactivity

CMR measurements of BA area at baseline correlated to IVUS measurements (21.3 ± 5.8 versus 24.1 ± 6.6 mm², $r=0.87$, $P<0.01$). The CMR and IVUS measurements of BAR also correlated ($r=0.87$, $P<0.01$). Five of the 8 patients (62.5%) had coronary artery disease (triple-vessel coronary disease in 4 and isolated LAD stenosis in 1 patient). FMD by CMR was significantly less in patients with CAD compared with patients without CAD ($-3.6\pm2.4\%$ versus $5.0\pm1.7\%$, $P<0.01$), with no difference in GTN responses. The significance was not reproduced by IVUS ($-3.7\pm4.1\%$ versus $-1.0\pm5.4\%$, $P=0.22$) with the same sample size. There were no significant differences between non-CAD and CAD patients with regard to total cholesterol (5.4 ± 0.7 and 5.3 ± 0.6 mmol/L, $P=0.42$), mean blood pressure (107 ± 18 and 98 ± 8 mm Hg, $P=0.16$), and age (55 ± 6 years versus 60 ± 10 years, $P=0.23$).

In the 8 patients assessed by CMR, there was significant noncircularity of the BA cross-section with major and minor diameters of 4.8 ± 0.9 and 4.3 ± 0.6 mm ($P<0.01$). The noncircularity was confirmed by IVUS.

The BAR measured by IVUS correlated with EXUS ($r=0.57$, $P<0.05$). Using EXUS in the same sample size, we were not able to demonstrate a significant reduction in FMD

in CAD patients compared with non-CAD patients ($0.5\pm0.7\%$ versus $1.9\pm2.5\%$, $P=0.29$).

Reproducibility and Repeatability of CMR for Assessment of Arterial Reactivity

The mean difference between 2 blinded measurements (intraobserver variability) of area and FMD by CMR with the same observer (M.S.) was 0.00 ± 0.14 mm² and $0.29\pm1.5\%$, respectively. The mean difference between blinded measurements (interobserver variability) of BA area and FMD by 2 observers (M.S. and A.E.) was 0.04 ± 0.21 mm² and $0.48\pm2.2\%$, respectively. Test-retest repeatability (interstudy reproducibility) was significantly better by CMR than by EXUS (Table 2). The more reproducible assessment of FMD by CMR results in the need for smaller sample sizes when FMD change is assessed. We calculated that to perform our study using EXUS diameter and EXUS area would have required a total of 158 and 672 subjects, respectively.

Subjects for DMPA Study

One control subject decided to start DMPA after the first CMR examination and was withdrawn from the rest of the study. One long-term user of DMPA decided not to have her repeat injection after the first scan and was subsequently withdrawn from the rest of the study.

DMPA Study

FMD was significantly reduced in DMPA users compared with controls during menstruation ($1.1\pm3.0\%$ versus $8.0\pm4.8\%$, $P<0.01$, Figure 2), with no differences in GTN responses ($40\pm10\%$ versus $42\pm18\%$, $P=0.38$). FMD differences between the two groups were paralleled by differences in S-estradiol levels (57.8 ± 31.3 versus 95.6 ± 30.1 pmol/L, $P<0.01$). Comparing FMD and S-estradiol levels before and at peak concentration of circulating MPA in DMPA users with repeated measurements (n=12), changes corresponded without significant changes of both variables (Table 4). In controls with 2 measurements (n=9), FMD was significantly less in the menstrual phase compared with midcycle, with no effect on GTN responses, and the change in FMD was again paralleled by changes in S-estradiol levels (Table 4). In the entire sample size of healthy premenopausal women and correlating all corresponding data sets, S-estradiol levels correlated significantly to FMD ($r=0.43$, $P<0.01$). Three DMPA users (23%), but none of the controls, experienced symptoms linked to estrogen deficiency (Table 1).

Discussion

Long-term use of DMPA results in endothelial dysfunction, which may result from hypoestrogenism. Both FMD, an indicator of endothelial function, and S-estradiol were significantly reduced in users of DMPA compared with menstruating controls. To our knowledge, this is the first investigation of the effects of DMPA, and indeed any contraceptive progestogen, on vascular reactivity in young women. A similar study showed no negative endothelial effects of estrogen-progestogen oral contraceptives.[20] Whether these findings can be extrapolated to other ovulation-inhibiting progestogen-only contraceptive methods and correspond to

an increase in cardiovascular morbidity requires additional study.

Estrogens display a variety of their cardiovascular effects via the endothelium.[5] Increased production of endothelial NO has been accredited to estrogen-induced genomic and nongenomic activation of nitric oxide synthase.[21] FMD increases with estrogen replacement after the menopause,[22] and FMD reduction has been shown with drug-induced hypoestrogenism (GnRH agonist) in healthy premenopausal women.[19] Our results suggest that the level of S-estradiol is a determinant of endothelial function in healthy premenopausal women. DMPA users presented with lower cholesterol levels than controls, which may be a direct effect of DMPA.[23] It is possible that DMPA-induced metabolic changes may have had adverse effects on the endothelium in our study.

The estrogen levels in DMPA users were in the postmenopausal range, similar to other reports.[1] DMPA-induced hypoestrogenism is of concern with regard to osteoporosis risk,[4] and loss of bone mass with DMPA has been demonstrated.[24] However, no study has demonstrated increase in fracture incidence in DMPA users. Our results do not provide direct evidence that DMPA is linked to CAD. Limited epidemiological evidence of progestogen contraception has not demonstrated such an effect.[25] A case-control study reported no difference in odds ratio (OR) of progestogen use in cardiovascular disease.[25] However, only 37 cases and 122 controls used injectable progestogen (about 1% of the study population), limiting the power of the conclusions that may be drawn from this study. Additionally, most users of progestogen-only contraception used oral preparations, where hypoestrogenism is probably less pronounced, because contraception is achieved mainly via endometrial and cervical effects. It may be important to differentiate cardiovascular risk in users of progestogen-only contraception dependent on intrinsic estrogen levels. The incidence of cardiovascular disease in cases and controls with hypertension was significantly greater in users of injectable progestogen than in nonusers (OR 7.2; CIs, 1.32 to 38.7). Although our study reports important observations, additional evidence is required to establish causality.

This is the first report of measurement of arterial function by CMR. The validation study demonstrated that CMR and IVUS area measurements correlate well, whereas IVUS and EXUS measurements correlate less well. Direct measurement of brachial artery area by CMR may provide more reliable information than diameter measurements by EXUS. The percentage change in brachial artery area by CMR was greater than the percentage diameter change measured by EXUS given the same stimulus, thus making it a direct and more sensitive measure for detecting smaller changes. The one-directional measurement error by EXUS is low, but when assessing derived area change, the error associated with noncircularity of the artery is magnified. This may explain why the magnitude of change was larger by EXUS-derived area than by CMR-measured area. The test-retest repeatability of CMR was superior to that of EXUS in the same group of subjects. The coefficient of variability (CV) of FMD by CMR was lower than that reported before with EXUS. The EXUS repeatability in this report was at the lower end of values often reported; however, the CV was comparable with that reported by other groups.[26] We have previously reported better repeatability of EXUS measurements by another operator (mean interstudy difference, $1.40\pm0.95\%$),[18] and differences in repeatability have also been reported from other centers (CV of FMD responses, 0.62 and 1.28, respectively).[26,27] The latter variation in repeatability with EXUS might be related to selection of subjects and confounding factors, but might also be related directly to the imaging technique. Measurement of cross-sectional area by CMR versus diameter by EXUS avoids the inherent problem of identifying an exact diameter of a blood vessel, thus improving the repeatability.[13] The cross section of the brachial artery is not always circular but often oval (Figure 1), raising the possibility that dilatation may not occur uniformly in all directions. At least in part, these factors may explain differences between the techniques. The improved repeatability using CMR allows smaller sample sizes for research studies.

Limitations

Study data were acquired in a small number of subjects and in a nonrandomized fashion because of the obvious ethical problems of using placebo in a contraceptive study. The application of CMR to measure arterial reactivity is limited by cost and availability of equipment; however, the improved sensitivity and repeatability will enhance data quality and reliability in comparison with EXUS.

Conclusions

Long-term use of DMPA for contraception is linked to impaired brachial artery endothelial function and hypoestrogenism. These findings may have clinical implications, in particular with regard to the prevailing recommendation to use DMPA in women with cardiovascular disease.

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