Use of injectable hormonal contraception and women's risk of herpes simplex virus type 2 acquisition: a prospective study of couples in Rakai, Uganda

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Summary

Background The injectable hormonal contraceptive depo-medroxyprogesterone acetate (DMPA) has been associated with increased risk of HIV acquisition, but findings are inconsistent. Whether DMPA increases the risk of other sexually transmitted viral infections is unknown. We assessed the association between DMPA use and incident herpes simplex virus type 2 (HSV2) infection in women.

Methods In this prospective study, we enrolled HIV-negative and HSV2-negative women aged 15–49 years whose HIV-negative male partners were concurrently enrolled in a randomised trial of male circumcision in Rakai, Uganda. We excluded women if either they or their male partners HIV seroconverted. The primary outcome was HSV2 seroconversion, assessed annually. The male circumcision trial was registered with ClinicalTrials.gov, number NCT00425984.

Findings Between Aug 11, 2003, and July 6, 2006, we enrolled 682 women in this study. We noted HSV2 seroconversions in 70 (10%) women. Incidence was 13·5 per 100 person-years in women consistently using DMPA (nine incident infections per 66·5 person-years), 4·3 per 100 person-years in pregnant women who were not using hormonal contraception (18 incident infections per 423·5 person-years), and 6·6 per 100 person-years in women who were neither pregnant nor using hormonal contraception (35 incident infections per 529·5 person-years). Women consistently using DMPA had an adjusted hazard ratio for HSV2 seroconversion of 2·26 (95% CI 1·09–4·69; p=0·029) compared with women who were neither pregnant nor using hormonal contraception. Of 132 women with HSV2-seropositive partners, seroconversion was 36·4 per 100 person-years in consistent DMPA users (four incident infections per 11 person-years) and 10·7 per 100 person-years in women who were neither pregnant nor using hormonal contraception (11 incident infections per 103 person-years; adjusted hazard ratio 6·23, 95% CI 1·49–26·3; p=0·012).

Interpretation Consistent DMPA use might increase risk of HSV2 seroconversion; however, study power was low. These findings should be assessed in larger populations with more frequent follow-up than in this study, and other contraceptive methods should also be assessed. Access to a wide range of highly effective contraceptive methods is needed for women, particularly in sub-Saharan Africa.

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Introduction

Hormonal contraceptive use is becoming increasingly common in sub-Saharan Africa,[1] and long-acting injectable hormonal contraceptives are some of the most effective and frequent contraceptive methods used by African women.[2,3] During the past two decades, depo-medroxyprogesterone acetate (DMPA), the most widely used injectable contraceptive method,[3] has been associated with increased risk of HIV acquisition by women in some but not all studies.[4] A possible association between DMPA use and HIV risk has prompted debate over its safety and relative reproductive health benefits, with some people calling for randomised controlled trials of various contraceptive methods, including DMPA.[5] Whether DMPA increases risk of other sexually transmitted viral infections such as herpes simplex virus type 2 (HSV2) is unknown.

Investigators of many observational studies have assessed the association between injectable and oral hormonal contraceptive use and HIV acquisition in women,[4] although interpretation of results has been complex.[5,6] In a systematic review, Polis and colleagues[4] identified little evidence for increased HIV risk with use of either oral hormonal contraception or norethisterone injectable hormonal contraception; however, results conflicted as to whether DMPA was linked with HIV acquisition. Of seven high-quality epidemiological studies that reported DMPA-specific estimates and were included

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Research in context

Evidence before this study

We did a systematic search of PubMed with no specified start date up to Jan 23, 2015, using the following key terms: "hormonal", "contraception", "contraceptives progesterone", "progestin", "progestins", "oral contraception", "oral contraceptives", "depo(t)medroxyprogesterone", "depo", "depot", "DMPA", "NET EN", "NET-EN", "norethisterone enanthate", "medroxyprogesterone 17-acetate", "injectable", "levonorgestrel", "etonogestrel", "implant", "uniplanar", "jadelle", "implanon", "norplant", "norplant2", "sino-implant", "contraceptives postcoital", "emergency contraception and contraceptives", "ulipristal acetate", "Plan B", "mifepristone", "levonorgestrel intrauterine devices", "IUD", "IUCD", "IUS", "intrauterine system", "intra-uterine system", "intrauterine device", "intra-uterine device", "Mirena", "Cyclofem", "Lunell", "Mesigyna", "Cyclo Provera", "Cycloprovera", "contraceptive devices", "contraceptive agents and ring", "NuvaRing", "Nuva Ring", "contraceptive devices/agents and patch", "Ortho Evra", "Ortho-Evra", "herpes simplex", "herpes genitalis", "genital herpes", "herpes simplex virus infection", "HSV", "HSV type 2", "genital herpes infection", "herpes simplex virus type 2", and "HSV2". We identified only one study that assessed the relation between incident HSV2 infection and use of either oral or injectable hormonal contraception in women. Investigators of this study of HIV-negative sex workers in Kenya found no association between use of injectable or oral hormonal contraception and HSV2; however, the HSV2 serostatus of the women's partners was unknown. Findings from challenge studies in mice suggest that DMPA might increase susceptibility to HSV2 in the genital tract.

Added value of this study

This study is the first prospective study to assess the relation between use of depo-medroxyprogesterone acetate (DMPA), a widely used injectable hormonal contraceptive method, and incident HSV2 infection in HIV-negative women with male partners of known HSV2 status. Despite few incident events in a small number of women and a long interval between annual study visits, we noted significantly increased HSV2 risk in women who self-reported consistently using DMPA.

Implications of all the available evidence

Taken together with previous evidence, these findings suggest that incident HSV2 might be associated with use of DMPA, which should be assessed in other studies with larger sample sizes and more frequent follow-up than in this study. Improved access to a wide range of highly effective contraceptive methods should be a public health priority, particularly in sub-Saharan Africa.

in the review, investigators of three noted significantly increased HIV risk,[7–9] two noted non-significantly increased risk,[10,11] and two noted non-significantly decreased risk[12,13] in DMPA users. A meta-analysis[14] including these and three other studies estimated a 40% increased HIV risk with DMPA use for all women (pooled hazard ratio [HR] 1·40, 95% CI 1·16–1·69), although this risk was lower for women in the general population (excludes known high-risk women; 1·31, 1·10–1·57).

The pathophysiology of how DMPA, a progestin-only hormonal contraceptive method, might theoretically increase HIV risk is unclear. Thinning of the vaginal epithelium and endometrium, increased immune activation in the genital tract, and heightened risk of other sexually transmitted infections, including HSV2, have been postulated as mechanisms.[15,16] Vaginal thinning after administration of DMPA has been noted in rhesus macaques;[17,18] however, investigators of studies in women have found little evidence for vaginal thinning or cervical ectopy with DMPA use.[19–21] Findings from other studies suggest that progestins might modulate HIV susceptibility via increased expression of CCR5 coreceptors on CD4-positive T cells and recruitment of HIV target cells (eg, dendritic cells) to the vaginal and stromal epithelial tissues.[22–24]

HSV2 is a common sexually transmitted virus, and its transmission is associated with substantial morbidity around the world.[25,26] The worldwide burden of HSV2, like that of HIV, is disproportionately concentrated in sub-Saharan Africa.[27] HSV2 infection is a frequent cause of genital ulcer disease,[28,29] which is a risk factor for HIV.[30,31] The main HSV2 target cells in the genital tract are the epithelial cells, although HSV2 can infect dendritic cells.[25,32–34] Findings from challenge studies in mice have shown increased HSV2 susceptibility and impaired immunological responses to HSV2 in the presence of DMPA.[35,36] Despite high HIV and HSV2 co-infection,[31,37] investigators of only one epidemiological study[38] have examined the association between use of oral or injectable hormonal contraception and incident HSV2 infection in women. The investigators found no association between use of injectable or oral hormonal contraceptives and HSV2 in HIV-negative sex workers in Kenya; however, the HSV2 serostatus of the women's partners was unknown. In this study, we assessed whether use of DMPA or oral hormonal contraceptives was associated with increased risk of HSV2 acquisition in HIV-negative women in long-term sexual partnerships with HIV-negative men of known HSV2 serostatus in Rakai, Uganda.

Methods

Study design and participants

The Rakai Health Sciences Program in Rakai, Uganda, enrolled HIV-negative men into a randomised trial of male circumcision for prevention of HIV and other sexually transmitted infections.[39,40] Men were eligible for enrolment if they were HIV negative, uncircumcised, aged 15–49 years, and provided written informed consent.

Men who had contraindications for surgery (eg, anaemia or active genital infection) were treated, and if their medical disorder resolved, they were rescreened and enrolled into the trial. Those with anatomical abnormalities (eg, hypospadias), other medical contraindications, or indications for surgery (eg, severe phimosis) were excluded. Participants were randomly assigned to receive immediate male circumcision (intervention group) or male circumcision delayed for 24 months (control group). Consenting female partners of male trial participants who were married or in long-term consensual relationships were invited to participate in a separate follow-up study.[41,42]

The analysis in our study was restricted to HIV-negative and HSV2-negative women, aged 15–49 years, who were enrolled concurrently with their HIV-negative male partner. Female participants needed to have at least one follow-up visit at which we ascertained their HSV2 status, and her partner's HIV status also needed to be known. We excluded women if either they or their male partners HIV seroconverted because an association exists between HIV and HSV2 acquisition,[30,31,43] and these events were too infrequent for stratified analyses. Women who self-reported use of intrauterine devices or implants were excluded because small numbers of these women limited power to detect associations between their use and HSV incidence.

The trials were approved by the Uganda National Council for Science and Technology (Kampala, Uganda), the Science and Ethics Committee of the Uganda Virus Research Institute (Entebbe, Uganda), the Committee for Human Research at Johns Hopkins University Bloomberg School of Public Health (MD, USA), and the Western Institutional Review Board (WA, USA).[40,41] Participants provided written informed consent, which included permission to use samples for future research. The male circumcision trial was registered with ClinicalTrials.gov, number NCT00425984, and the follow-up of female partners of the male circumcision trial participants was also registered, number NCT00124878.

Procedures

At enrolment and two annual follow-up visits, we interviewed the women to ascertain sociodemographic characteristics, sexual risk behaviours, and health status. We similarly interviewed the men. We also asked the women about pregnancy status and intention to use and actual use of family planning methods, including oral, injectable, and implantable hormonal contraceptives. Because DMPA is the main injectable hormonal contraceptive method available in Rakai (more than 98% of users; Gray RH, unpublished), we did not ask women to specify which form of injectable contraception that they were using. At each visit, we asked the men and women to provide blood samples, which we maintained at 4–10°C for less than 6 h, and then at –80°C until assayed. The primary study outcome was HSV2 seroconversion.

We established HSV2 infection with HSV2 ELISA (Kalon Biological, Guildford, UK). As previously described,[37,39,42,44] we defined an HSV2 seroconversion as a negative enrolment serology (optical density of 0·9 or less) followed by a positive follow-up serology (optical density index value of at least 1·5). We confirmed all incident HSV2-positive cases ascertained with ELISA with Euroimmun Western blot (Euroimmun, Lubeck, Germany).[37,39,42] We established HIV status with two separate ELISAs and confirmed discordant results with an HIV1 western blot. We established HSV2 and HIV1 infection status for the women and their partners.[40]

We assessed self-reported hormonal contraceptive use at each study visit. We treated hormonal contraceptive use as a time-varying exposure, and classified use during a given visit interval with report of hormonal contraceptive use at the present and previous study visits. We defined hormonal contraceptive use during a visit interval as either DMPA or oral hormonal contraceptive use. We analysed consistency of hormonal contraceptive use in DMPA users. We classified DMPA use as consistent when a woman reported using DMPA at both her present and previous study visit. When a woman reported using DMPA at her present but not previous visit, we classified her as having initiated use during that interval. We classified women who self-reported using DMPA at the previous but not present visit as having discontinued use. We defined women who were pregnant at the present or previous visit and who reported no hormonal contraceptive use—DMPA or oral hormonal contraception—as a separate exposure group. The reference group was women who were neither pregnant nor using any hormonal contraceptive method at the present or previous study visit, and included women who were using condoms, natural family planning methods, or spermicides, or had a hysterectomy or tubal ligation.

Statistical analysis

We established significant differences in enrolment demographics, sexual behaviours, and health characteristics between women who ever reported oral or DMPA use and those who never reported hormonal contraceptive use using a χ^2 test for categorical variables and a Wilcoxon rank-sum test for continuous variables.

We administratively censored women who did not acquire HSV2 at year 2 or at loss to follow-up. We included all women in the primary analysis, irrespective of their spouse's HSV2 status. In a separate analysis, we assessed the association between DMPA use and HSV2 acquisition in women whose male partner was HSV2 seropositive at her present study visit, including women whose partners acquired HSV2 during the same visit interval.

We calculated time at risk as the time elapsed between annual visits. We assumed that women who seroconverted between visits did so at the midpoint of the visit interval. We calculated incidence of HSV2 per 100 person-years. In view of the discrete nature of the

data (only two follow-up visits) and the large amount of time between annual visits, we used complementary log-log regression models with generalised estimating equations and robust variance estimators to estimate HRs and 95% CIs. The complementary log-log model is a discrete time survival model and is similar to the continuous time Cox proportional hazards model; however, the discrete model assumes constant hazard within an interval, although the hazard can vary between intervals.[45] We used generalised estimating equations and robust variance estimators to obtain population-averaged effect estimates and account for correlation between findings from the same woman over time.[46] We used the complementary log-log regression models for all primary analyses and the sensitivity analysis that excluded women who self-reported condom use. We implemented the models using the *xtcloglog* command with the *pa* and *robust* options in Stata version 11.0.

We included variables in adjusted analyses if we either identified them as confounders in previous observational studies of hormonal contraceptive use and HIV acquisition and not as a marker of HSV2 or HIV1 infection[6] or associated them with hormonal contraceptive exposure and HSV2 seroconversion in the study population at p<0·1. Time-invariant covariates included age and educational status of female participants and their male partners, and female self-reported number of lifetime sexual partners at enrolment. Time-varying confounders included male circumcision, female self-reported coital frequency, and female and male partner self-report of any condom use and non-marital partners in the past year. We did not include coital frequency or male circumcision status in the final adjusted model because they did not significantly alter the primary effect estimates (by more than 10%) or improve the model likelihood in nested comparisons as established by the quasi-likelihood under independence criterion.[47]

Role of the funding source

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Aug 11, 2003, and July 6, 2006, 4996 HIV-negative men were enrolled into the trial of male circumcision.[39,40] 1638 HIV-negative female partners were enrolled and followed up concurrently with their male partners in a separate follow-up study.[41,42] Of these women, 740 HSV2-negative and HIV-negative women were concurrently enrolled with their HIV-negative male spouse. After excluding 41 (6%) women with insufficient follow-up, two (<1%) who used intrauterine devices, five (1%) who used implantable contraception, six (1%) who HIV seroconverted, and four (1%) whose partners HIV seroconverted, we included 682 (92%) women with 660 male partners in the final analysis population. The

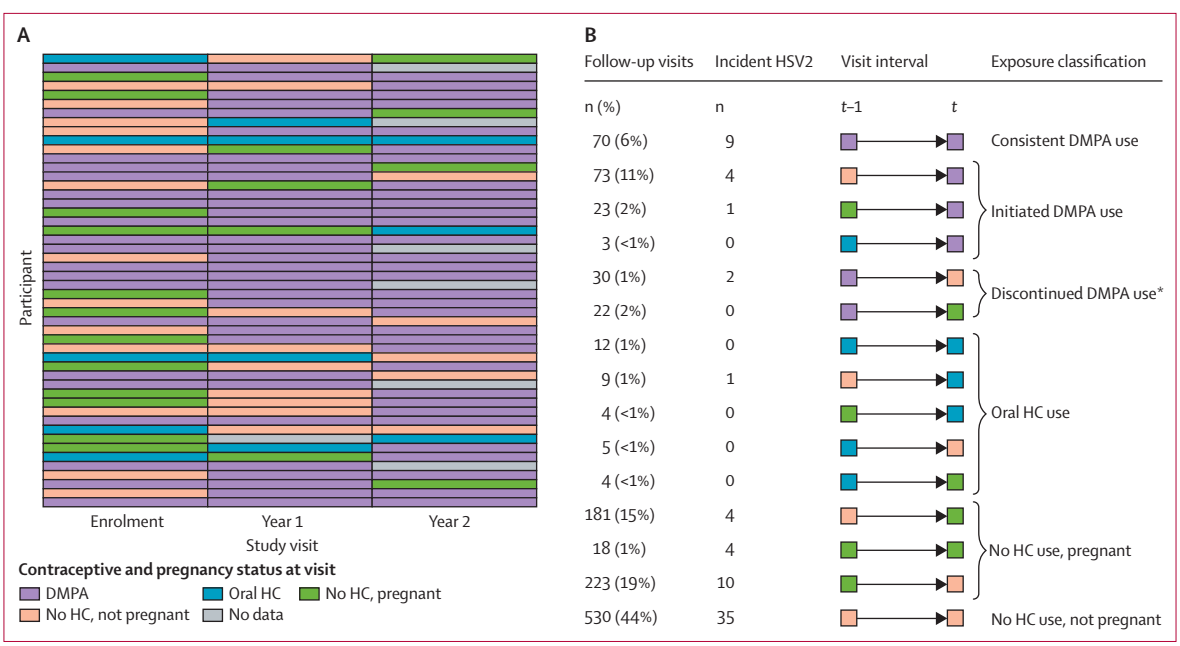

Figure: **Patterns of hormonal contraceptive use over 2 years and HSV2 incidence**
(A) Individual-level patterns of hormonal contraceptive use between enrolment and year 2 study visits for a random sample of 50 hormonal contraceptive users. (B) Classification of exposure to hormonal contraception for a visit interval using contraceptive and pregnancy statuses from the present and previous study visits. DMPA=depo-medroxyprogesterone acetate. HC=hormonal contraception. HSV2=herpes simplex virus type 2. *t*–1=previous visit. *t*=present visit. *No woman switched from DMPA to oral HC.

number of female participants exceeded that of male partners because of polygamous relationships. The number of women with male partners in the intervention (n=341) and control (n=341) arms of the male circumcision trial was equal. Female participants contributed 1207 person-visits to the analysis, and we followed them up for an average of 1·7 person-years. Retention was 95% at year 1 (650 participants) and 82% at year 2 (557 participants). Follow-up between year 1 and year 2 did not significantly differ between oral hormonal contraceptive users (16 [84%] of 19), DMPA users (consistent use 21 [72%] of 29; initiated use 44 [83%] of 53; discontinued use 18 [67%] of 27), or pregnant women (206 [84%] of 244) when we compared these groups with women who were neither pregnant nor using hormonal contraception (232 [78%] of 296).

173 (25%) of 682 women self-reported ever using hormonal contraception at enrolment or at a follow-up visit. Of these women using hormonal contraception, 147 (85%) reported DMPA use only, 22 (13%) reported oral hormonal contraceptive use only, and four (2%) reported DMPA and oral use at different study visits. 129 (85%) of 151 women initiated (99 [66%]) or discontinued (52 [34%]) DMPA use during the study (figure). Only 57 (38%) of 151 women consistently reported DMPA use at successive visits. Pregnancy was common—338 (50%) of female participants fell pregnant at either enrolment (163 [24%]) or a follow-up visit (218 [32%]).

Table 1 shows participant characteristics at enrolment, broken down by contraceptive use. For all women, the median age of female participants was 24 years (IQR 21–28) and the median age of their male partners was 28 years (IQR 24–32). Only 112 (16%) of women reported using condoms at enrolment. Self-report of extramarital partners was 19 (3%) among women and 225 (33%) in men. We noted some differences in enrolment characteristics between women who did and did not use DMPA or oral hormonal contraception. Women who reported DMPA use were slightly older and less likely to be pregnant than were those who reported no hormonal contraceptive use, and women who either reported DMPA or oral hormonal contraceptive use were slightly more educated than were those who reported no hormonal contraceptive use. Their male partners were also slightly more educated if their female partner had either reported DMPA or oral hormonal contraceptive use, and slightly more likely to report using condoms in the past year if their female partner had reported DMPA use than were those whose female partner had reported no hormonal contraceptive use. At enrolment, both DMPA and oral hormonal contraceptive users and non-users had similar proportions of HSV2-seropositive partners, self-reported condom use, and self-reported genital ulcer disease. Male partners of DMPA and oral hormonal contraceptive users and non-users also had similar self-reported genital ulcer disease and assignment to the intervention arm of the male circumcision trial.

	No HC	Any oral HC*	p value†	Any DMPA*	p value†
Overall (n=682)					
n (%)	509 (75%)	26 (4%)	..	151 (22%)	..
Demographics at enrolment					
Female age (years)	24 (21–27)	25 (21–27)	0·874	25 (22–29)	0·023
Male partner's age (years)	27 (24–32)	30 (25–33)	0·633	29 (25–33)	0·085
Polygamous male partner	55 (11%)	4 (15%)	0·467	19 (13%)	0·543
Female education status					
None	67 (13%)	2 (8%)		13 (9%)	
Primary	379 (74%)	16 (62%)		98 (65%)	
Secondary	57 (11%)	6 (23%)		30 (20%)	
Postsecondary	6 (1%)	2 (8%)	0·011	10 (7%)	<0·0001
Male partner's educational status					
None	51 (10%)	1 (4%)		6 (4%)	
Primary	374 (73%)	12 (46%)		97 (64%)	
Secondary	65 (13%)	8 (31%)		29 (19%)	
Postsecondary	19 (4%)	5 (19%)	<0·0001	19 (13%)	<0·0001
Sexual behaviours at enrolment					
Female lifetime sexual partners					
One	234 (46%)	9 (35%)		61 (40%)	
Two	177 (35%)	9 (35%)		53 (35%)	
Three	65 (13%)	5 (19%)		23 (15%)	
Four or more	33 (6%)	3 (12%)	0·488	14 (9%)	0·455
Non-marital relationships in the past year	17 (3%)	0	0·344	2 (1%)	0·193
Condom use in the past year‡	79 (16%)	4 (15%)	0·978	30 (20%)	0·214
Male partner sexual behaviours at enrolment					
Non-marital relationships in the past year	163 (32%)	5 (19%)	0·170	58 (38%)	0·144
Condom use in the past year‡	159 (31%)	6 (23%)	0·379	68 (45%)	0·002
Health characteristics					
Ever pregnant during study	269 (53%)	11 (42%)	0·294	61 (40%)	0·007
Female self-reported GUD at enrolment	42 (8%)	0	0·127	12 (8%)	0·905
Male partner self-reported GUD at enrolment	23 (5%)	0	0·268	8 (5%)	0·691
Male partner in trial intervention group	256 (50%)	14 (54%)	0·724	73 (48%)	0·674
Male partner's HSV2 status at enrolment					
Negative	366 (72%)	21 (81%)		116 (77%)	
Indeterminate	61 (12%)	2 (8%)		12 (8%)	
Positive	80 (16%)	3 (12%)		22 (15%)	
Unknown	2 (<1%)	0	0·792	1 (1%)	0·496

Data are n (%) or median (IQR). HC=hormonal contraception. DMPA=depo-medroxyprogesterone acetate. GUD=genital ulcer disease. HSV2=herpes simplex virus 2. *Four women self-reported using oral HC and DMPA during the study (not at same visits). The four women are included in both groups—women using any oral HC and women using any DMPA. †Wilcoxon rank-sum p values for continuous variables and χ^2 p values for categorical variables (compared with non-HC users). ‡214 (98%) of 218 women reporting condom use at enrolment or follow-up reported consistent condom use during sexual intercourse.

Table 1: **Baseline characteristics**

26 (4%) women reported using DMPA or oral hormonal contraception at the start of a visit interval and were then found to be pregnant at their next visit (figure). 16 of these women reported planning to become pregnant during the interval. Of those who had an unplanned pregnancy

	Incident infections/py	Incidence per 100 py	Unadjusted HR	p value	Adjusted HR	p value
HC use status						
No HC use, not pregnant	35/529·5	6·6	1·00	..	1·00	..
No HC use, pregnant	18/423·5	4·3	0·64 (0·36–1·12)	0·119	0·66 (0·37–1·19)	0·169
Oral HC use	1/35·5	2·8	0·44 (0·06–3·22)	0·418	0·49 (0·08–3·01)	0·441
DMPA use						
Discontinued use	2/51·0	3·9	0·57 (0·14–2·37)	0·437	0·58 (0·13–2·51)	0·535
Initiated use	5/96·5	5·2	0·75 (0·30–1·92)	0·554	0·75 (0·29–1·92)	0·632
Consistent use	9/66·5	13·5	2·02 (0·96–4·26)	0·066	2·26 (1·09–4·69)	0·029
Age (years)*						
Male partner	..	..	0·95 (0·90–1·00)	0·043	0·95 (0·86–1·04)	0·247
Female participant	..	..	0·98 (0·94–1·03)	0·400	1·00 (0·92–1·09)	0·992
Female educational status						
None	5/151·5	3·3	0·49 (0·20–1·21)	0·165	0·66 (0·29–1·50)	0·323
Primary	58/88·5	6·8	1·00	..	1·00	..
Secondary	6/164·0	3·7	0·55 (0·24–1·28)	0·165	0·41 (0·16–1·05)	0·065
Postsecondary	1/31·5	3·2	0·50 (0·07–3·49)	0·488	0·15 (0·02–1·27)	0·083
Male educational status						
None	4/104·0	3·8	0·82 (0·29–2·30)	0·688	1·09 (0·39–3·11)	0·863
Primary	42/854·5	4·9	1·00	..	1·00	..
Secondary	18/171·0	10·5	2·19 (1·26–3·81)	0·005	1·92 (1·09–3·41)	0·024
Postsecondary	6/73·0	8·2	1·71 (0·74–3·97)	0·212	2·85 (1·13–7·18)	0·026
Female lifetime sexual partners						
One	24/549·0	4·4	1·00	..	1·00	..
Two	22/415·5	5·3	1·23 (0·69–2·20)	0·486	1·27 (0·71–2·29)	0·414
Three	11/161·5	6·8	1·58 (0·77–3·26)	0·212	1·41 (0·66–3·01)	0·371
Four or more	13/76·5	17·0	3·99 (2·03–7·82)	<0·001	3·45 (1·72–6·92)	<0·0001
Female non-marital relationships						
No	65/1162·0	5·6	1·00	..	1·00	..
Yes	5/40·5	12·3	2·26(0·96–5·29)	0·062	1·28 (0·46–3·58)	0·633
Male non-marital relationships						
No	46/918·5	5·0	1·00	..	1·00	..
Yes	24/284·0	8·5	1·68 (1·03–2·76)	0·039	1·08 (0·61–1·90)	0·802
Female condom use						
No	54/973·0	5·6	1·00	..	1·00	..
Yes	16/216·5	7·4	1·33 (0·77–2·33)	0·337	0·89 (0·49–1·63)	0·715
Male condom use						
No	36/822·5	4·4	1·00	..	1·00	..
Yes	33/371·5	8·9	2·00 (1·25–3·21)	0·004	1·70 (0·98–2·97)	0·060

Data are n/py or HR (95% CI). py=person-years. HR=hazard ratio. HC=hormonal contraception. DMPA=depo-medroxyprogesterone acetate. *We treated age as a conterminous variable in this model.

Table 2: **Risk factors for incident HSV2 infection**

(ten), one reported using oral hormonal contraception at the time that she became pregnant and one reported using DMPA. The remaining eight reported using no form of contraception. When a woman became pregnant and was not using hormonal contraception at her previous visit (199 [16%] of 1207), 108 (54%) of these pregnancies were reported as unplanned. Of these women, none reported using hormonal contraception—DMPA or oral hormonal contraception—at the time that they became pregnant.

As shown in table 2, we noted incident HSV2 infections in 70 (10%) women (primary outcome). 39 (56%) infections occurred in women with HSV2-seronegative male partners, 23 (33%) occurred in women with HSV2-seropositive partners, and eight (11%) occurred in women who had partners of unknown or indeterminate HSV2 status. 13 (19%) women who seroconverted reported genital ulcers in the past year (HR 2·2, 95% CI 1·2–4·0). Overall, HSV2 incidence was 5·8 per 100 person-years. We noted no increased risk of HSV2 acquisition during

pregnancy (adjusted HR 0·66, 95% CI 0·37–1·19) or in oral hormonal contraceptive users (0·49, 0·08–3·01). Risk of HSV2 was significantly increased in women consistently using DMPA (2·26, 1·09–4·69; p=0·029); however, we did not note heightened risk in those who recently initiated or discontinued DMPA use (table 2). In a sensitivity analysis, results were similar in women who never reported condom use (appendix).

Other factors that were significantly associated with female HSV2 seroconversion after adjustment included an increased number of female lifetime sexual partners at enrolment and increased male partner's educational status (table 2). Few women reported non-marital relationships during follow-up (35 [5%] of 682). Of the HSV2-seroconverting women who had HSV2-seronegative partners, two (5%) of 39 reported non-marital relationships during the interval between the last negative and first positive visits.

132 (19%) women had an HSV2-seropositive spouse at enrolment or follow-up. Of these women, 27 (20%) had partners who HSV2 seroconverted after enrolment, including six (5%) HSV2-incident women whose spouses seroconverted during the same visit interval. The rate of female HSV2 acquisition was 10·6 per 100 person-years (23 per 218 person-years) in women with an HSV2-infected partner, which was significantly higher than was incidence in women with an HSV2-negative partner (4·8 per 100 person-years; 39 per 814·5 person-years; p=0·001). Incidence of HSV2 in women with HSV2-infected partners is shown in table 3. The increased risk of incident HSV2 in consistent DMPA users with HSV2-infected partners was statistically significant.

	Incident infections/py	Incidence per 100 py	Unadjusted HR	p value	Adjusted HR	p value
No HC use, not pregnant	11/103·0	10·7	1·00	..	1·00	..
No HC use, pregnant	3/71·5	4·2	0·38 (0·11–1·35)	0·133	0·27 (0·06–1·13)	0·075
Oral HC use	0/6·0	..	..	..	..	..
DMPA use						
Discontinued use	1/7·5	13·3	1·19 (0·16–9·01)	0·863	2·42 (0·29–20·1)	0·411
Initiated use	4/19·0	21·1	1·86 (0·59–5·78)	0·283	1·42 (0·25–8·08)	0·691
Consistent use	4/11·0	36·4	3·31 (0·99–11·0)	0·051	6·23 (1·49–26·3)	0·012

Data are n/py or HR (95% CI). Analysis included six women whose partners also herpes simplex virus 2 seroconverted during the same visit interval; one of these women was consistently using DMPA. py=person-years. HR=hazard ratio. HC=hormonal contraception. DMPA=depo-medroxyprogesterone acetate.

Table 3: **Association between hormonal contraceptive use and HSV2 acquisition in HIV-negative women with HSV2-seropositive partners**

Discussion

Consistent use of DMPA was associated with a greater than two times increased risk of HSV2 seroconversion compared with no hormonal contraceptive use. In an analysis restricted to women with known exposure to HSV2-seropositive partners, the risk of incident HSV2 with consistent DMPA use was more than six times greater than with no hormonal contraceptive use. Pregnancy was common, with half of women falling pregnant during the 2 year follow-up, predominately in those not using any form of contraception. Investigators of some, but not all, observational studies have noted increased risk of HIV during pregnancy,[48] including one prospective study in Rakai.[49] We noted no such analogous risk of HSV2 acquisition during pregnancy in this study.

Most women who reported using a hormonal contraceptive method used DMPA, and DMPA use was highly dynamic. DMPA use has been associated with HIV acquisition in several studies, including a meta-analysis,[14] but the data are inconsistent. Two studies assessed the association between DMPA use and HIV in Rakai;[50,51] however, investigators of both noted no significantly increased HIV risk in DMPA users. By contrast with DMPA, little evidence exists that oral hormonal contraceptive methods augment HIV risk.[4] We had very low power to detect an association between oral hormonal contraceptive use and HSV2 acquisition, although our results suggest that risk of HSV2 acquisition is not increased in oral hormonal contraceptive users.

We cannot rule out the possibility that unmeasured or poorly measured behaviours, or other factors associated with DMPA use and a cause of HSV2 infection, could have confounded our results.[6] For example, if women with high-risk sexual behaviours were more likely to use DMPA but these behaviours were not reported accurately, the findings could be biased. Indeed, women substantially under-reported extramarital partnerships, as evidenced by half of all HSV2 infections occurring in women who reported no external relationships but had HSV2-seronegative partners. However, we still noted an association between DMPA and incident HSV2 when we restricted analyses to those women with known HSV2-infected partners only.

Consistent condom use can reduce HIV acquisition by 80%;[52] however, findings from meta-analyses[53] suggest that condom effectiveness is only 30% for HSV2 acquisition. This decreased effectiveness is because HSV2 can be transmitted to and from genital areas that are not protected by male condoms.[25] Although condoms have been identified as important time-dependent confounders in observational studies of HIV and hormonal contraceptive use,[6] we noted no association between female reported condom use and HSV2 acquisition. We also noted no significant differences in our effect estimates in a sensitivity analysis excluding women who ever reported using condoms.

This study has other limitations. Information about contraceptive exposures was based on self-reported use of DMPA or oral hormonal contraceptive use at the time of a study visit, and we inferred consistency of DMPA use by reported use at successive visits. The trial from which we derived these data was not designed to assess associations between contraception and HSV2 acquisition, and the interval between follow-up visits (1 year) cannot accurately assess the timing of HSV2 acquisition in relation to

See Online for appendix

contraceptive exposures. The long follow-up intervals could also affect women's recall of their risk behaviours. However, the prospective design, laboratory methods, and inclusion of information about both women and their stable male partners were study strengths. Notably, the main finding of increased risk of HSV2 seroconversion with consistent DMPA use was based on only nine incident cases and the CIs around the point hazards estimate are wide. Nevertheless, other studies of HIV acquisition and injectable contraception were also based on similarly small numbers of seroconversions.[54]

The finding that consistent use of DMPA might increase women's risk of HSV2 acquisition might be relevant to the associations between HIV risk and use of DMPA in some previous studies,[4] and might show that DMPA broadly affects the risk of viral sexually transmitted infections. However, these results are based on only a small number of cases and need to be substantiated in other populations with more frequent follow-up than in this study and validated contraceptive exposure histories. Future randomised trials of hormonal contraceptives should assess incidence of HSV2 in addition to HIV as potential study outcomes.[5] The possibility of an increased risk of HIV and HSV2 associated with DMPA use should be weighed against its benefits, including reduced risk of maternal mortality and unwanted pregnancy. Increased access to other forms of highly effective, long-acting, low-dose contraceptive methods, including intrauterine devices and implants, is needed in sub-Saharan Africa.

Contributors

MKG, RHG, FM, and AART conceptualised and designed the study. GK, FN, JK, SJR, DS, RHG, and MJW oversaw survey data collection. AART, TCQ, and ADR oversaw and did laboratory assays and contributed reagents. MKG did statistical analyses. FM and TL assisted with data management and organisation. All authors wrote the report and agreed with the results and conclusions.

Declaration of interests

We declare no competing interests.

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