Effect of circumcision of HIV-negative men on transmission of human papillomavirus to HIV-negative women: a randomised trial in Rakai, Uganda

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Summary

Background **Randomised trials show that male circumcision reduces the prevalence and incidence of high-risk human papillomavirus (HPV) infection in men. We assessed the efficacy of male circumcision to reduce prevalence and incidence of high-risk HPV in female partners of circumcised men.**

Methods **In two parallel but independent randomised controlled trials of male circumcision, we enrolled HIV-negative men and their female partners between 2003 and 2006, in Rakai, Uganda. With a computer-generated random number sequence in blocks of 20, men were assigned to undergo circumcision immediately (intervention) or after 24 months (control). HIV-uninfected female partners (648 of men from the intervention group, and 597 of men in the control group) were simultaneously enrolled and provided interview information and self-collected vaginal swabs at baseline, 12 months, and 24 months. Vaginal swabs were tested for high-risk HPV by Roche HPV Linear Array. Female HPV infection was a secondary endpoint of the trials, assessed as the prevalence of high-risk HPV infection 24 months after intervention and the incidence of new infections during the trial. Analysis was by intention-to-treat. An as-treated analysis was also done to account for study-group crossovers. The trials were registered, numbers NCT00425984 and NCT00124878.**

Findings **During the trial, 18 men in the control group underwent circumcision elsewhere, and 31 in the intervention group did not undergo circumcision. At 24-month follow-up, data were available for 544 women in the intervention group and 488 in the control group; 151 (27·8%) women in the intervention group and 189 (38·7%) in the control group had high-risk HPV infection (prevalence risk ratio=0·72, 95% CI 0·60–0·85, p=0·001). During the trial, incidence of high-risk HPV infection in women was lower in the intervention group than in the control group (20·7 infections *vs* 26·9 infections per 100 person-years; incidence rate ratio=0·77, 0·63–0·93, p=0·008).**

Interpretation **Our findings indicate that male circumcision should now be accepted as an efficacious intervention for reducing the prevalence and incidence of HPV infections in female partners. However, protection is only partial; the promotion of safe sex practices is also important.**

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Introduction

Infection with human papillomavirus (HPV) is common in sexually active individuals, especially in developing countries.[1] HPV infection can cause genital warts, and high-risk genotypes cause penile and anal cancers in men, and cervical cancer in women.[1,2] Cervical cancer is the third most common cancer in women worldwide.[3] More than 85% of the HPV disease burden is in developing countries, and cervical cancer is the leading cause of cancer mortality in women in east Africa.[3]

Three randomised trials, done in Uganda,[4] Kenya,[5] and South Africa,[6] showed that male circumcision substantially decreased the risk of HIV acquisition in men. These trials reported that male circumcision also reduced the prevalence of penile high-risk HPV infection by about 35%,[7,8] reduced the acquisition of new high-risk HPV infections, and increased clearance of pre-existing high-risk HPV infection in men without HIV infection.[9] We have also reported[10] that, compared with female partners of uncircumcised men, female partners of circumcised men had lower rates of genital ulcer disease, *Trichomonas vaginalis* infection, and bacterial vaginosis. However, male circumcision did not reduce the rate of HIV transmission from men with HIV to their female partners.[11]

In several observational studies, female partners of circumcised men were found to have a substantially reduced risk of cervical neoplasia,[12–14] but findings are not consistent.[15,16] Many of these observational studies were small, were vulnerable to confounding by sexual behaviours in men and women, and assessed male circumcision status by self-report or the female partners' report, which could have affected the validity of data. Moreover, the long latent interval between initial infection with high-risk HPV and the development of cervical neoplasia complicates causal inferences from retrospective data for previous

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exposures. Because infection with high-risk HPV is a necessary precondition for cervical neoplasia,[17] the potential efficacy of male circumcision for the prevention of cervical neoplasia can best be assessed in randomised controlled trials of male circumcision that measure incidence, prevalence, and clearance of high-risk HPV infection in female partners of men randomly assigned circumcision immediately or after a delay. In this study, we assessed the effectiveness of male circumcision to prevent high-risk HPV infection in HIV-negative female partners of HIV-negative men who were enrolled in two randomised controlled trials of male circumcision in Rakai, Uganda.

Methods

Study design and participants

Two parallel but independent trials of male circumcision for the prevention of HIV and other sexually transmitted infections were done in Rakai, Uganda, as previously described.[4,7,10,11,18] Both trials began enrolment of participants in September, 2003. The first trial enrolled 4996 uncircumcised men who did not have HIV infection and were between 15 and 49 years old. The primary aim was to assess the effectiveness of male circumcision for the prevention of HIV infection; secondary aims were to assess the effect on other sexually transmitted infections (including HPV infection) in men. The trial was stopped in December, 2006, after an interim analysis showed that male circumcision was effective for the prevention of HIV acquisition in men. The second trial enrolled 922 men with HIV infection and 600 men without HIV infection, all of whom were between the ages of 15 and 49 years, and the female partners of male participants in both trials. It aimed primarily to assess the efficacy of male circumcision for the prevention of HIV transmission to female partners.[10,11] Secondary outcomes were the transmission of other sexually transmitted infections (including HPV infection) to female partners. In December, 2006, the conditional power to detect 60% efficacy against HIV infection in women, as specified in the study protocol, was calculated to be only 4·9% and no more participants were enrolled.[11] Follow-up of female partners was completed in December, 2007.

In both studies, men were eligible for enrolment if they were uncircumcised, aged 15–49 years, had no medical indications or contraindications for male circumcision, and provided written informed consent. Male circumcision was done with the sleeve procedure, which is safe.[4,19]

Men who were married or in long-term consensual relationships were asked to identify their female partners, who were contacted separately and invited to participate in a follow-up study. Women were eligible for enrolment if they provided informed consent and their male partner was a trial participant. The consent form described study procedures, risks, benefits, and the voluntary nature of participation. After providing written informed consent, women were enrolled and followed up at 12 months and 24 months.

Men and women who had HIV infection at enrolment, or who acquired HIV infection during the trial, were excluded from analysis because HIV infection increases the risk of persistent HPV.[20] During the course of the trial, more men in the control group than in the intervention group seroconverted to HIV;[4] inclusion of HIV seroconverters in analyses could therefore have biased HPV results. To ascertain a woman's baseline HPV status immediately before her partner was circumcised, and to avoid potential bias between groups, analysis was restricted to women who enrolled at the same time as their male partners. All participants were offered free voluntary HIV counselling and testing as individuals or as couples, provided with education about the prevention of HIV and sexually transmitted infections, and offered free condoms. Women received US$1·50 per visit as compensation for their time and effort.

The trials were approved by four institutional review boards: the Science and Ethics Committee of the Uganda Virus Research Institute (Entebbe, Uganda); the HIV subcommittee of the National Council for Research and Technology (Kampala, Uganda); the Committee for Human Research at Johns Hopkins University, Bloomberg School of Public Health (Baltimore, MD, USA); and the Western Institutional Review Board (Olympia, WA, USA). The trial funded by the Bill & Melinda Gates Foundation was overseen by an independent Data and Safety Monitoring Board. The other trial was overseen by the National Institutes of Health Vaccine and Prevention Data and Safety Monitoring Board. A community advisory board provided suggestions on the conduct of the trials and recommended the amount of compensation per study visit.

Randomisation and masking

Men were randomly assigned to undergo immediate circumcision (intervention group) or circumcision after 24 months (control group). Randomisation was done in blocks of 20 and stratified by community,[4,11] with computer-generated random numbers prepared by the study statistician at Johns Hopkins University, who had no contact with participants. Each participant selected an opaque envelope that contained the written allocation of assignment from a stack of 20. Clinical officers (equivalent to a physician's assistant) enrolled participants, administered the randomisation process, and did follow-up visits. Laboratory technicians and female fieldworkers were masked to male participants' circumcision status.

Procedures

At each study visit, women were interviewed by female interviewers to ascertain sociodemographic characteristics, sexual risk behaviours, and their health status, including symptoms of genital-tract infections (genital ulcer disease, vaginal discharge, and dysuria). Symptomatic women were treated appropriately by nurses or clinical officers in local clinics. At each visit, women were asked to provide self-administered vaginal swabs for HPV detection.[21] They were

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instructed to squat, insert a 20 cm Dacron or cotton-tipped swab, and to rotate the swab high in the vaginal vault. A fieldworker collected the swab samples and stored them in specimen transport medium (Digene Corporation, Gaithersburg, MD, USA). The specimens were maintained at 4–10°C for less than 6 h until they were frozen at –80°C. Studies[22,23] have shown that self-collected vaginal swabs are as effective as physician-collected cervical swabs for HPV detection.

Detection of the β-globin gene was used to identify the presence of cellular DNA from desquamated vaginal epithelium to assess adequacy of sample collection. Swabs without amplifiable cellular or viral DNA were not included in the analyses; only those vaginal samples with detectable β-globin, HPV, or both were included in the primary analysis. HPV genotyping was done with the Roche HPV Linear Array (Roche Diagnostics, Indianapolis, IN, USA), as previously

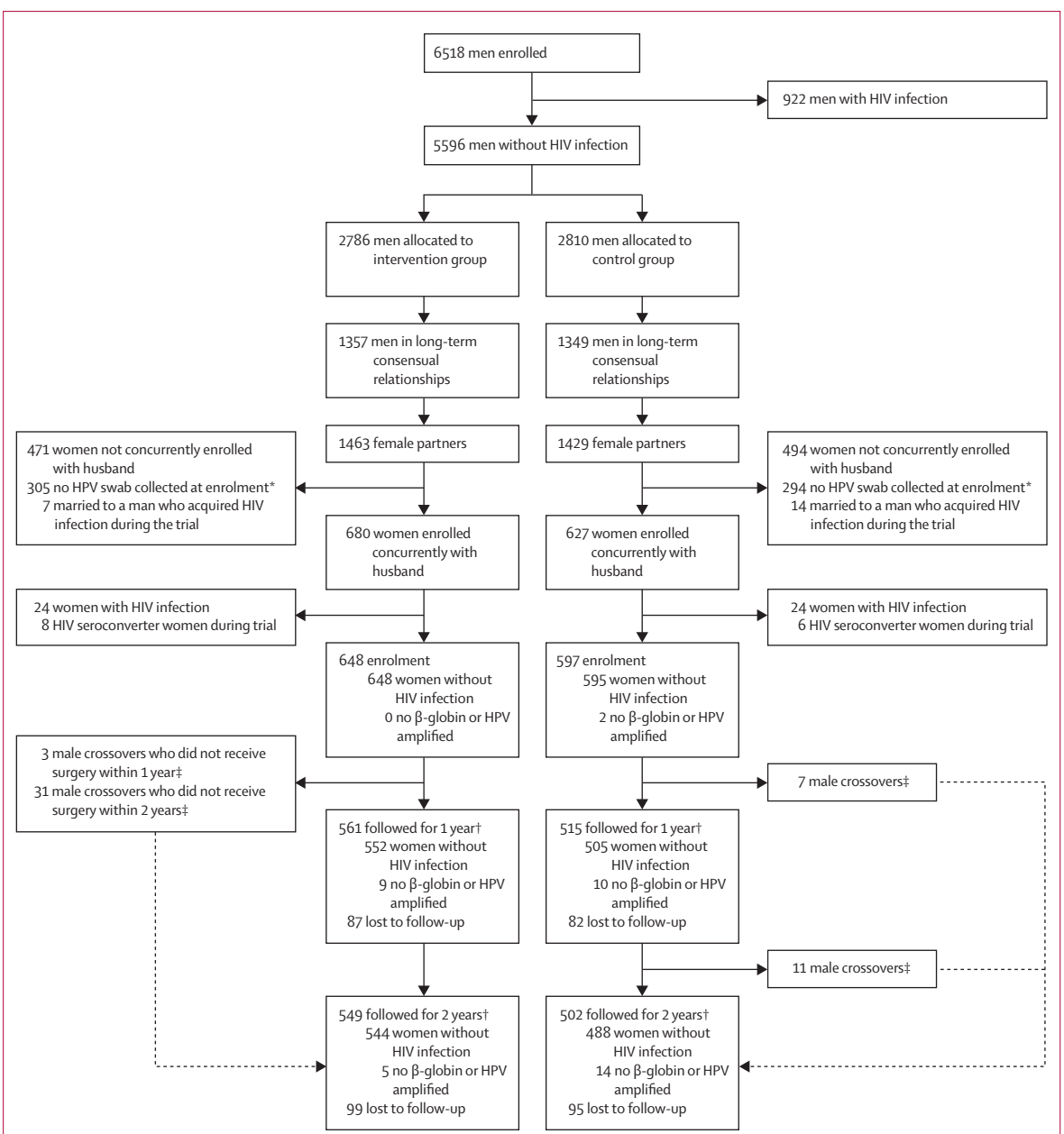

Figure: **Trial profile**
HPV=human papillomavirus. *Because of HPV Digene swab stock outage. †Some individuals were unavailable for follow-up at year 1 but were available for follow-up in year 2. ‡ The intention-to-treat analysis included all women who had detectable β-globin or HPV. The male crossovers were only relevant to the as-treated-analysis. Three women at year 2 were excluded because their male partner's circumcision status could not be confirmed at that visit.

	Men		Female partners	
	Intervention group (n=598)	Control group (n=561)	Intervention group (n=648)	Control group (n=597)
Age (years)				
15–19	8 (1%)	9 (2%)	80 (12%)	65 (11%)
20–24	121 (20%)	118 (21%)	208 (32%)	208 (35%)
25–29	173 (29%)	178 (32%)	178 (27%)	174 (29%)
30–49	296 (49%)	256 (46%)	182 (28%)	150 (25%)
Marital status				
Not married	1 (<1%)	0	1 (<1%)	0
Monogamous	524 (88%)	506 (90%)	529 (82%)	506 (85%)
Polygynous union	73 (12%)	55 (10%)	118 (18%)	91 (15%)
Religion				
Catholic	384 (64%)	378 (67%)	391 (60%)	362 (61%)
Protestant	172 (29%)	146 (26%)	186 (29%)	158 (26%)
Saved/Pentecostal/other	38 (6%)	35 (6%)	57 (9%)	62 (10%)
Muslim	4 (<1%)	2 (<1%)	14 (2%)	15 (3%)
Education				
None	51 (9%)	50 (9%)	93 (14%)	78 (13%)
Primary	437 (73%)	420 (75%)	464 (72%)	444 (74%)
Secondary	84 (14%)	69 (12%)	80 (12%)	65 (11%)
Post-secondary	26 (4%)	22 (4%)	11 (2%)	10 (2%)
Number of sexual partners in previous year				
0	0	0	0	2 (<1%)
1	343 (57%)	305 (54%)	625 (96%)	573 (96%)
>2	255 (43%)	256 (46%)	23 (4%)	22 (4%)
Non-marital relationships in previous year				
No	497 (83%)	469 (84%)	641 (99%)	585 (98%)
Yes	101 (17%)	92 (16%)	7 (1%)	12 (2%)
Condom use in previous year				
None	398 (67%)	359 (64%)	554 (85%)	485 (81%)
Inconsistent	193 (32%)	194 (35%)	92 (14%)	107 (18%)
Consistent	7 (1%)	8 (1%)	2 (<1%)	3 (<1%)
Alcohol use with sex in previous year	320 (54%)	333 (60%)	221 (34%)	196 (33%)
Transactional sexual intercourse in previous year*	5 (<1%)	8 (1%)	5 (<1%)	3 (<1%)
Previous receipt of voluntary counselling and testing	167 (28%)	119 (21%)	146 (23%)	117 (20%)
Self-reported symptoms of STIs in previous year				
Genital ulcer disease	54 (9%)	51 (9%)	87 (13%)	83 (14%)
Urethral or vaginal discharge	25 (4%)	24 (4%)	298 (46%)	283 (47%)
Dysuria	33 (6%)	42 (7%)	126 (19%)	126 (21%)

Data are number (%). STI=sexually transmitted infection. *Sexual intercourse in exchange for money or gifts. Condom use, the use of alcohol with sexual intercourse, and transactional sexual intercourse were assessed only in sexually active individuals, although the percentages in these categories were calculated on the basis of the total number of individuals enrolled in each study group.

Table 1: **Baseline characteristics, risk behaviours, and symptoms of sexually transmitted infections of men and their female partners, by study group**

described.[24] The high-risk HPV genotypes assessed were genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. HIV status was established with two separate ELISAs, and discordant results were confirmed by HIV-1 western blot analysis, as previously described.[4]

Primary outcomes were the prevalence of high-risk HPV infection in female partners at 12 and 24 months after intervention and the incidence of new infections during the course of the trial.

Statistical analysis

The sexually transmitted infection endpoints were specified in the original protocols for the trials. However, we did not write a specific protocol for the secondary analysis of the HPV endpoints because it was specified in the HPV grant proposal to the National Institutes of Health, and no protocol for this HPV secondary endpoint was requested.

Enrolment and follow-up characteristics, sexual risk behaviours, and symptoms of sexually transmitted

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infections were tabulated by study group and differences were assessed by χ^2 tests. To assess the efficacy of male circumcision for reduction of HPV infection in women we used an intention-to-treat analysis. To account for group crossovers, an as-treated analysis was also done. Intervention-group crossovers were classified as uncircumcised if the male partner did not accept surgery. Control-group crossovers were classified as circumcised if the male partner underwent male circumcision from another source. HPV prevalence was tabulated by follow-up year (ie, year 1 or year 2), and the prevalence risk ratios (PRR) and 95% CI of HPV in the intervention group relative to the control group were estimated with modified Poisson regression.

Incident HPV was defined as a newly detected genotype identified in women whose swab was negative for any HPV at the previous study visit, or women who had previously had a positive result for HPV but had at least one newly detected HPV genotype during the next follow-up period. HPV incidence rates per 100 person-years were estimated; we assumed that a new HPV infection was acquired at the midpoint of the follow-up interval during which the new infection was detected. New HPV detection during the follow-up intervals used an individual participant as the unit of observation, and each woman with a newly detected HPV genotype was counted only once per follow-up interval, irrespective of whether one or several HPV genotypes were detected. Incidence was classified as single or multiple (two or more) new HPV genotype-specific infections. The incidence of genotype-specific high-risk HPV was assessed with women-intervals as the unit of observation, and the population at-risk was women without that genotype at a previous study visit, irrespective of coinciding infection with other high-risk HPV genotypes. The incidence of high-risk HPV infection in each group was assessed by baseline sociodemographic and behavioural covariates. Incidence rate ratios (IRR) and 95% CI of new high-risk HPV detection in the intervention versus control group at 12 months and 24 months were estimated by use of Poisson log-linear regression with the logarithm of person-years as the offset. Because some of the male partners were polygynous, Poisson regression with generalised estimating equation[25] exchangeable correlation structure was used to model the incidence rate ratio, to account for the potential correlation between women sharing the same partner.

Clearance (ie, loss of detection) of high-risk HPV was estimated in women with pre-existing high-risk HPV genotype-specific infections, and high-risk HPV genotype was the unit of observation. Clearance was expressed as the proportion of pre-existing high-risk HPV genotype-specific infections that were negative for that genotype at a subsequent study visit. Clearance was assessed for each high-risk HPV genotype, and all genotype-specific clearance events were combined to provide overall estimates. The clearance risk ratio (RR) of any high-risk HPV genotype infection was estimated with a log binomial model with robust variance estimates based on generalised estimating equations to account for multiple clearance events in women with more than one initial high-risk HPV infection.

A predetermined minimum detectable PRR of female partners of men in intervention versus control by study population was 0·70 with a known sample size, assuming a power (1–β=0·8) and two-tailed α=0·05. The rate ratio was calculated with the likelihood ratio test for testing the inequality of two proportions, that is, H_0: $p_1/p_2=1$, versus H_1: $p_1/p_2<1$ (p_1=intervention, p_2=control). The calculation was done with PASS 2008 software. For incidence and clearance, potential confounders were examined in univariate analyses, and covariates shown to be associated at α<0·20, or suspected confounders on the basis of

	Intervention group HPV positive/N* (%)	Control group HPV positive/N* (%)	Prevalence risk ratio (95% CI)
All genotypes			
Baseline	359/648 (55%)	334/595 (56%)	0·99 (0·89–1·09)
Year 1	293/552 (53%)	302/505 (60%)	0·89 (0·80–0·99)
Year 2	251/544 (46%)	277/488 (57%)	0·81 (0·72–0·92)
Low-risk HPV			
Baseline	267/648 (41%)	249/595 (42%)	0·98 (0·86–1·12)
Year 1	220/552 (40%)	222/505 (44%)	0·91 (0·79–1·05)
Year 2	180/544 (33%)	209/488 (43%)	0·77 (0·66–0·90)
High-risk HPV			
Baseline	228/648 (35%)	221/595 (37%)	0·95 (0·82–1·10)
Year 1	187/552 (34%)	209/505 (41%)	0·82 (0·70–0·96)
Year 2	151/544 (28%)	189/488 (39%)	0·72 (0·60–0·85)

*The total number of women (N) includes all those who had detectable β-globin, human papillomavirus (HPV), or both. Individuals were included in both the high-risk category and the low-risk category if they had both human papillomavirus (HPV) genotypes.

Table 2: **HPV prevalence in women at enrolment, and at 12 months and 24 months follow-up, by male circumcision study group (intention-to-treat analysis)**

	Male circumcised group HPV positive/N* (%)	Male not circumcised group HPV positive/N* (%)	Prevalence risk ratio (95% CI)
All genotypes			
Baseline	359/648 (55%)	334/595 (56%)	0·99 (0·89–1·09)
Year 1	278/528 (53%)	317/529 (60%)	0·88 (0·79–0·98)
Year 2	247/521 (47%)	279/508 (55%)	0·86 (0·77–0·97)
Low-risk HPV			
Baseline	267/648 (41%)	249/595 (42%)	0·98 (0·86–1·12)
Year 1	213/528 (40%)	229/529 (43%)	0·93 (0·81–1·07)
Year 2	178/521 (34%)	209/508 (41%)	0·83 (0·71–0·97)
High-risk HPV			
Baseline	228/648 (35%)	221/595 (37%)	0·95 (0·82–1·10)
Year 1	172/528 (33%)	224/529 (42%)	0·77 (0·66–0·90)
Year 2	148/521 (28%)	192/508 (38%)	0·75 (0·63–0·90)

*The total number of women (N) includes all those who had detectable β-globin, human papillomavirus (HPV), or both. Three women at year 2 were excluded because their male partner's circumcision status could not be confirmed at that visit. Individuals could be included in both the high-risk and the low-risk HPV categories if they had both HPV genotypes.

Table 3: **HPV prevalence in women at enrolment, and at 12 months and 24 months follow-up, by male circumcision status (as-treated analysis)**

	Intervention group		Control group		Incidence rate ratio (95% CI)
	N/person-years	Incidence/ 100 person-years	N/person-years	Incidence/ 100 person-years	
Any low-risk HPV Infection					
0–1 year	143/480·5	29·8	151/428·5	35·2	0·84 (0·67–1·06)
1–2 year	103/428·5	24·0	125/355·5	35·2	0·68 (0·53–0·89)
Total 0–2 year	217/909	23·9	225/784	28·7	0·83 (0·69–1·00)
Single low-risk HPV Infection					
0–1 year	108/480·5	22·5	103/428·5	24·0	0·94 (0·71–1·22)
1–2 year	68/428·5	15·9	80/355·5	22·5	0·71 (0·51–0·97)
Total 0–2 year	133/909	14·6	114/784	14·5	1·01 (0·78–1·29)
Multiple low-risk HPV Infection					
0–1 year	35/480·5	7·3	48/428·5	11·2	0·65 (0·42–1·01)
1–2 year	35/428·5	8·2	45/355·5	12·7	0·65 (0·41–1·00)
Total 0–2 year	84/909	9·2	111/784	14·2	0·65 (0·49–0·87)
Any high-risk HPV Infection					
0–1 year	121/491·5	24·6	148/430	34·4	0·72 (0·56–0·91)
1–2 year	94/433	21·7	103/366·5	28·1	0·77 (0·58–1·02)
Total 0–2 year	191/924·5	20·7	214/796·5	26·9	0·77 (0·63–0·93)
Single high-risk HPV Infection					
0–1 year	88/491·5	17·9	101/430	23·5	0·76 (0·57–1·01)
1–2 year	58/433	13·4	61/366·5	16·6	0·80 (0·56–1·15)
Total 0–2 year	109/924·5	11·8	114/796·5	14·3	0·82 (0·63–1·07)
Multiple high-risk HPV Infection					
0–1 year	33/491·5	6·7	47/430	10·9	0·61 (0·39–0·96)
1–2 year	36/433	8·3	42/366·5	11·5	0·73 (0·46–1·13)
Total 0–2 year	82/924·5	8·9	100/796·5	12·6	0·71 (0·53–0·95)

HR=high risk. LR=low risk. N=the number of women with newly detected human papillomavirus (HPV) infections.

Table 4: **HPV incidence in women by study group and follow-up interval for participants with amplifiable cellular or viral DNA at sequential study visits**

biological reasoning or previous studies were included in multivariate regression analyses. All p values are two-sided. Analyses were done with R 2.8.1 and SAS 9.2 (SAS Institute, Cary, NC, USA). The trials were registered, numbers NCT00425984 (funded by National Institutes of Health) and NCT00124878 (funded by the Bill & Melinda Gates Foundation).

Role of the funding source

The Bill & Melinda Gates Foundation maintained oversight of trial progress, and participated in open review by the data safety and monitoring board and in the interpretation of data for the trial of HIV transmission to female partners of circumcised men. For the assessment of HPV transmission, the sponsors of the study had no role in study design or data collection, analysis, or interpretation. The corresponding author had final responsibility for the decision to submit for publication.

Results

The trial profile is given in the figure. Of the 2786 men in the intervention group who did not have HIV infection at enrolment, 1357 (49%) reported that they were married or in a consensual relationship with a total of 1463 women (mean=1·08 female partners per man). Of the 2810 men in the control group who did not have HIV infection at enrolment, 1349 (48%) reported that they were married or in a consensual relationship with a total of 1429 women (mean=1·06 female partners per man).

Because of a temporary lack of HPV Digene swabs and sample transport media, 305 women in the intervention group and 294 women in the control group did not have a vaginal swab obtained at enrolment. After exclusion of women who had HIV infection, did not enrol with their male partner, and who could not provide a vaginal swab at enrolment, 648 women were enrolled in the intervention group and 597 were enrolled in the control group.

At the 12-month follow-up, data were available for 561 (86·6%) women in the intervention group and 515 (86·3%) women in the control group. At the 24-month follow-up, data were available for 549 (84·7%) women in the intervention group and 502 (84·1%) women in the control group. The approach to specimen collection was well accepted, with compliance rates of more than 90% at baseline and follow-up visits (data not shown). Throughout

the duration of the study, 14 (0·8%) vaginal swab samples from women in the intervention group and 26 (1·6%) vaginal swab samples from women in the control group were excluded from the analyses because of the absence of detectable β-globin or HPV DNA (figure). This difference in DNA detection was significant (p=0·04). 34 men did not accept surgery by 1 year after randomisation (intervention-group crossovers). 18 men in the control group received male circumcision from other sources (seven in the first year and 11 in the second year after randomisation; control-group crossovers).

Baseline sociodemographic characteristics, sexual behaviours, and symptoms of sexually transmitted infections were much the same for men in the two study groups, except that a greater proportion of men in the intervention group had previously received voluntary counselling and testing and more men in the control group consumed alcohol before sexual intercourse (table 1). Baseline characteristics and HPV prevalences were much the same for women in the two study groups (table 1 and table 2). At year 2, the prevalence of high-risk HPV infection was higher in women in the control group than in women in the intervention group in both the intention-to-treat analysis (p=0·001; table 2) and the as-treated analysis (p=0·002; table 3). Between enrolment and year 2, the prevalence of high-risk HPV decreased by 7·4% (p=0·006) in the intervention group and increased by 1·6% (p=0·59) in the control group (table 2).

During the 2-year follow-up, the incidence of high-risk HPV infections was lower in the intervention group than in the control group (p=0·008; table 4). The incidence of low-risk HPV was also lower in the intervention group than in the control group (p=0·05; table 4). New detection of multiple high-risk HPV genotypes (p=0·02) and multiple low-risk HPV genotypes (p=0·003) was less likely for women in the intervention group than for those in the control group. Differences between the groups in the incidence of single high-risk HPV and low-risk HPV infections in women were not statistically significant (table 4).

The incidence of genotype-specific high-risk HPV infection during the 24-month follow-up was lower in women in the intervention group than in women in the control group for all high-risk HPV genotypes assessed, except HPV-39 (table 5). However, the differences were significant only for HPV genotypes 33, 35, and 58. We also assessed the incidence of low-risk HPV genotypes 6 and 11 because they are associated with genital warts; incidence of HPV-11 was lower in women in the intervention group than in women in the control group (p=0·006), but incidence of HPV-6 did not differ between the study groups (p=0·87; webappendix p 1).

The incidence of high-risk HPV infection per 100 person-years was lower in women in the intervention group than in women in the control group for all sociodemographic and behavioural subgroups, except women who reported non-marital relationships and symptoms of dysuria, but the differences were statistically significant only for a few subgroups (age 15–19 years or 25–29 years, monogamous, secondary education or beyond, 1 sex partner, non-marital relationships, no condom use in previous year, no alcohol with sex, no genital ulcers, no discharge, and no dysuria; table 6). After adjustment for enrolment characteristics (age, condom use, alcohol consumption with sex, and number of sex partners during the previous year), and control for correlation caused by polygynous relationships, the incidence of new high-risk HPV infections was lower in women in the intervention group than in women in the control group (IRR 0·77, 95% CI 0·65–0·92, p=0·004).

For all high-risk HPV genotypes together, clearance in women who had positive results for high-risk HPV infection was more likely in the intervention group than in the control group (p=0·014; table 7). After we adjusted for baseline age, education, number of sexual partners, alcohol consumption with sex, and condom use, and accounted for possible correlation of a woman clearing more than one genotype, cumulative high-risk HPV clearance was higher in women in the intervention group than it was in women in the control group (RR 1·10, 95% CI 1·03–1·20, p=0·003). Clearance at year 2 of high-risk HPV infections (with all genotypes) acquired during the first year of the trial was also more likely in women in the intervention group (82%, n=124) than in women in the control group (70%, n=127; RR=1·17, 95% CI 1·04–1·32, p=0·014). Clearance of genotype-specific low-risk HPV infections was also more likely in women in the intervention group than in women in the control group (webappendix p 2).

Self-reported rates of women's sexual practices and symptoms of sexually transmitted infections were assessed by the male partner's circumcision status (webappendix p 3). At year 1, we recorded no difference

See Online for webappendix

	Intervention group		Control group		Incidence rate ratio (95% CI)
	N/person-years	Incidence/ 100 person-years	N /person-years	Incidence/ 100 person-years	
HPV-16	36/939	3·8	40/848	4·7	0·81 (0·52–1·28)
HPV-18	37/974·5	3·8	34/854	4·0	0·95 (0·60–1·52)
HPV-31	15/1000·5	1·5	17/890·5	1·9	0·79 (0·39–1·57)
HPV-33	8/1006	0·8	26/868	3·0	0·27 (0·12–0·59)
HPV-35	23/971·5	2·4	35/867·5	4·0	0·59 (0·35–0·99)
HPV-39	18/996	1·8	13/894·5	1·5	1·24 (0·61–2·54)
HPV-45	18/991	1·8	21/880·5	2·4	0·76 (0·41–1·43)
HPV-51	42/956	4·4	48/834	5·8	0·76 (0·50–1·15)
HPV-52	23/973·5	2·4	32/857	3·7	0·63 (0·37–1·08)
HPV-56	29/984·5	2·9	28/879	3·2	0·92 (0·55–1·55)
HPV-58	23/977·5	2·4	35/838·5	4·2	0·56 (0·33–0·95)
HPV-59	27/992·5	2·7	33/866·5	3·8	0·71 (0·43–1·19)
HPV-66	24/967	2·5	29/871·5	3·3	0·75 (0·43–1·28)
HPV-68	18/980	1·8	26/877	3·0	0·62 (0·34–1·13)

N is the number of women with newly detected high-risk human papillomavirus (HPV) genotypes.

Table 5: **Genotype-specific female high-risk HPV incidence during 2 years, by study group**

	Intervention group		Control group		Incidence rate ratio (95% CI)
	N/person-years	High-risk HPV incidence/ 100 person-years	N/person-years	High-risk HPV incidence/ 100 person-years	
Age (years)					
15–19	21/101	20·8	29/75	38·7	0·54 (0·31–0·94)
20–24	68/294·5	23·1	73/263	27·8	0·83 (0·60–1·16)
25–29	48/260	18·5	66/236·5	27·9	0·66 (0·46–0·96)
30–49	54/269	20·1	46/222	20·7	0·97 (0·65–1·44)
Marital status					
Not married	1/1·5	66·7	0/0	0·0	
Monogamous	153/754	20·3	176/672	26·2	0·77 (0·62–0·96)
Polygamous	37/169	21·9	38/124·5	30·5	0·72 (0·46–1·13)
Education					
None	27/144	18·8	26/108	24·1	0·78 (0·45–1·34)
Primary	142/648·5	21·9	159/592	26·9	0·82 (0·65–1·02)
Secondary or beyond	22/132	16·7	29/96·5	30·1	0·56 (0·32–0·97)
Number of sexual partners*					
1	183/903	20·3	204/771	26·5	0·77 (0·63–0·94)
>2	8/21·5	37·2	9/22	40·9	0·91 (0·35–2·36)
Non-marital relationships*					
No	188/915	20·5	211/785	26·9	0·76 (0·63–0·93)
Yes	3/9·5	31·6	2/8	25·0	1·26 (0·21–7·56)
Condom use in previous year*					
No	162/797·5	20·3	170/658	25·8	0·79 (0·63–0·98)
Yes	29/127	22·8	43/135	31·9	0·72 (0·45–1·15)
Alcohol use with sexual intercourse*					
No	115/598	19·2	148/538	27·5	0·70 (0·55–0·89)
Yes	76/326·5	23·3	65/255	25·5	0·91 (0·66–1·27)
Genital ulceration					
No genital ulcers	160/800	20·0	182/684	26·6	0·75 (0·61–0·93)
Genital ulcers	31/124·5	24·9	32/112·5	28·4	0·88 (0·53–1·43)
Vaginal discharge					
No discharge	105/495	21·2	125/433	28·9	0·73 (0·57–0·95)
Discharge	86/429·5	20·0	89/363·5	24·5	0·82 (0·61–1·10)
Dysuria					
No dysuria	145/749	19·4	172/617·5	27·9	0·70 (0·56–0·87)
Dysuria	46/175·5	26·2	42/179	23·5	1·11 (0·74–1·70)

* Assessed only in individuals who were sexually active. N is the number of women with newly detected high-risk human papillomavirus (high-risk HPV) infections.

Table 6: **Incidence of high-risk HPV infections during 2-year follow-up, by enrolment, sociodemographic, and behavioural characteristics, and symptoms of sexually transmitted infections reported during the year before enrolment**

in self-reported behaviours or symptoms of sexually transmitted infections between women in the two groups (webappendix p 3). However, at year 2, the rate of female self-reported genital ulcer disease was lower in women in the intervention group than in women in the control group (p=0·046; webappendix p 3). Additionally, the proportion of women who reported inconsistent condom use during the second year of follow-up was higher in the control group than in the intervention group (p=0·022; webappendix p 3). We recorded no difference in the reported number of sexual partners, non-marital relationships, vaginal discharge, or dysuria between the two study groups at either year 1 or year 2 (webappendix p 3). We recorded no difference in the number of sexual partners, non-marital relationships, or condom use between men in either study group at enrolment or during follow-up (data not shown).

Discussion

Circumcision of adolescent and adult men in a rural Ugandan population significantly reduced the prevalence and incidence of both low-risk and high-risk HPV infections and increased clearance of high-risk HPV infections in their female partners. The efficacy of male

	Intervention group cleared/N (%)	Control group cleared/N (%)	Risk ratio (95% CI)
HPV-16	39/75 (52%)	40/54 (74%)	0·70 (0·54–0·92)
HPV-18	35/39 (90%)	26/51 (51%)	1·76 (1·32–2·35)
HPV-31	14/24 (58%)	9/23 (39%)	1·49 (0·81–2·75)
HPV-33	15/22 (68%)	26/41 (63%)	1·08 (0·74–1·55)
HPV-35	33/49 (67%)	22/37 (59%)	1·13 (0·81–1·58)
HPV-39	17/27 (63%)	14/21 (67%)	0·94 (0·62–1·44)
HPV-45	20/32 (63%)	15/31 (48%)	1·29 (0·82–2·03)
HPV-51	34/55 (62%)	42/64 (66%)	0·94 (0·72–1·24)
HPV-52	35/74 (74%)	30/49 (61%)	1·22 (0·92–1·61)
HPV-56	24/33 (73%)	20/29 (69%)	1·05 (0·76–1·45)
HPV-58	28/43 (65%)	33/66 (50%)	1·30 (0·94–1·80)
HPV-59	22/26 (85%)	23/39 (59%)	1·43 (1·05–1·95)
HPV-66	39/53 (74%)	22/36 (61%)	1·20 (0·89–1·64)
HPV-68	21/43 (49%)	17/32 (53%)	0·92 (0·59–1·44)
Total	376/568 (66%)	339/573 (59%)	1·12 (1·02–1·22)

The denominators (N) are individuals with the specified high-risk human papillomavirus (high-risk HPV) genotype who had amplifiable cellular or viral DNA at both enrolment and follow-up, and were positive for the genotype at enrolment.

***Table 7*: Clearance of pre-existing genotype-specific female high-risk HPV infections, by study group**

circumcision for reducing high-risk HPV prevalence in female partners during the 2-year follow-up was 28%. Our finding of lower prevalence of high-risk HPV infections in female partners of circumcised men than in female partners of uncircumcised men accords with observational studies that show lower rates of cervical cancer associated with male circumcision.[12–14]

Although this study did not assess cervical neoplasia, persistent high-risk HPV infection is a prerequisite for the development of cervical cancer.[17] Clearance of high-risk HPV was more likely in female partners of men in the intervention group than in those of control-group men, with the exception of HPV-16, which is the genotype most strongly associated with penile and cervical cancers.[1] However, decreased incidence and prevalence of high-risk HPV infection will probably reduce the long-term risk of cervical cancer for women with circumcised male partners.

The biological mechanism through which male circumcision could reduce HPV infection rates in female partners probably involves a reduction of penile HPV carriage. Observational studies[26,27] have shown that HPV detection varies by anatomical site, and that male circumcision is associated with decreased HPV detection at the urethra, coronal sulcus, and shaft.[28] Data from two trials showed that male circumcision reduced the prevalence of high-risk HPV by 34% at the urethra and 35% at the coronal sulcus in men who did not have HIV infection,[7,8] probably through reduced acquisition and increased clearance.[8] The degree of genotype-specific concordance within couples is high.[29] Therefore, reduced penile high-risk HPV infection can lead to decreased female incidence and increased clearance, probably by lowering the chances of re-infection.

This study has several limitations. Enrolment samples were not obtained from about 20% of women in each group because of temporary stock shortages, which reduced the study sample size and power. However, similar proportions of women in both groups could not provide vaginal swab samples, so the missing data should not have biased the efficacy estimates. Men and women enrolled in this trial did not have HIV infection at baseline and were in stable partnerships. The study population may therefore be a low-risk population of compliant participants. Thus, the findings might not be applicable to individuals with HIV infection or populations of women with a higher frequency of multiple sex partners. For the few women who reported extramarital relationships, we did not note a reduction in new HPV detection. Because these extramarital relationships could have occurred with men of different circumcision status, estimates of the efficacy of circumcision could have been biased towards the null. Additionally, because follow-up data were obtained once a year, incident infections that cleared before the next follow-up visit would have been missed, and, therefore, the time of clearance could not be estimated. The interpretation of HPV epidemiology also has inherent difficulties; newly detected HPV infections could be a combination of newly acquired infections, sampling variability, or reactivation of previous latent infections that were below the limits of assay detection. If male circumcision does not affect the risk of recurrence or reactivation, we could have underestimated the reduction of new infections.

The reduction in high-risk HPV infection in female partners of circumcised men suggests that male circumcision could contribute to prevention of female high-risk HPV infection and cervical neoplasia in resource-poor settings where vaccines are not available, or in individuals with high-risk HPV genotypes that are not covered by available HPV vaccines. Male circumcision has now been shown to decrease HIV, herpes simplex virus-2, and HPV infections and genital ulcer disease in men, and also HPV infection, trichomoniasis, and bacterial vaginosis and genital ulcer disease in their female partners.[4–6,30] Thus, male circumcision reduces the risk of several sexually transmitted infections in both sexes, and these benefits should guide public health policies for neonatal, adolescent, and adult male circumcision programmes. Along with previous trial results in men,[7,8] these findings indicate that male circumcision should now be accepted as an efficacious intervention for reducing heterosexually acquired high-risk and low-risk HPV infections in men who do not have HIV and in their female partners. However, our results indicate that protection is only partial; the promotion of safe sex practices is also important.

Contributors

MJW, DS, and RHG oversaw the design and conduct of the trial, and participated in all data analyses and in writing the report. AART and PEG oversaw the HPV assays, provided laboratory quality control, and participated in all data analyses and writing the report. GK was responsible for study conduct in the field. SW supervised the trial surgeons and assisted in interpretation of data. FN, FM, VS, and NS participated in study implementation and data analysis and interpretation. XK and MZC participated in statistical analysis and data management. SJR and TCQ provided technical assistance with laboratory procedures and interpretation of results. AEO and KPE did the laboratory assays and quality control. All authors contributed to the preparation of the paper and approved the final version.

Conflicts of interest

PEG received research funding from Roche Molecular Diagnostics, who manufacture the HPV genotyping test used in this study. The other authors declare that they have no conflicts of interest.

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