Circumcision in HIV-infected men and its effect on HIV transmission to female partners in Rakai, Uganda: a randomised controlled trial

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Summary

Background Observational studies have reported an association between male circumcision and reduced risk of HIV infection in female partners. We assessed whether circumcision in HIV-infected men would reduce transmission of the virus to female sexual partners.

Methods 922 uncircumcised, HIV-infected, asymptomatic men aged 15–49 years with CD4-cell counts 350 cells per μL or more were enrolled in this unblinded, randomised controlled trial in Rakai District, Uganda. Men were randomly assigned by computer-generated randomisation sequence to receive immediate circumcision (intervention; n=474) or circumcision delayed for 24 months (control; n=448). HIV-uninfected female partners of the randomised men were concurrently enrolled (intervention, n=93; control, n=70) and followed up at 6, 12, and 24 months, to assess HIV acquisition by male treatment assignment (primary outcome). A modified intention-to-treat (ITT) analysis, which included all concurrently enrolled couples in which the female partner had at least one follow-up visit over 24 months, assessed female HIV acquisition by use of survival analysis and Cox proportional hazards modelling. This trial is registered with ClinicalTrials.gov, number NCT00124878.

Findings The trial was stopped early because of futility. 92 couples in the intervention group and 67 couples in the control group were included in the modified ITT analysis. 17 (18%) women in the intervention group and eight (12%) women in the control group acquired HIV during follow-up (p=0·36). Cumulative probabilities of female HIV infection at 24 months were 21·7% (95% CI 12·7–33·4) in the intervention group and 13·4% (6·7–25·8) in the control group (adjusted hazard ratio 1·49, 95% CI 0·62–3·57; p=0·368).

Interpretation Circumcision of HIV-infected men did not reduce HIV transmission to female partners over 24 months; longer-term effects could not be assessed. Condom use after male circumcision is essential for HIV prevention.

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Introduction

Three trials of male circumcision in HIV-negative men, including one undertaken in Rakai, Uganda, showed that circumcision reduced male acquisition of HIV by 50–60%;[1–3] as a result, male circumcision is now a recommended strategy for HIV prevention in men.[4] As these prevention programmes are scaled up, it is inevitable that men who are infected with HIV will also request to be circumcised, partly to avoid stigmatisation. We previously reported that male circumcision was safe and reduced rates of genital ulcer disease in asymptomatic HIV-infected men with CD4-cell counts 350 cells per μL or more.[5,6] In view of the social considerations and clinical findings, WHO and UNAIDS have recommended that surgery should not be denied to HIV-infected men who request the procedure unless there are medical contraindications.[4]

A previous observational study in HIV-discordant couples in Rakai suggested a lower rate of male-to-female HIV transmission from circumcised HIV-infected men, particularly if their viral load was below 50 000 copies per mL.[7] Two other observational studies also reported an association between male circumcision and reduced risk of HIV infection in female sexual partners.[8,9]

In parallel to the trial of male circumcision in HIV-uninfected men in Rakai,[3] we undertook a randomised controlled trial of male circumcision in HIV-infected men and enrolled their female partners. Trial objectives were to assess the safety of circumcision in HIV-infected men and the efficacy of male circumcision for the prevention of sexually transmitted infections (STIs) in HIV-infected men (reported elsewhere),[5,6] and to test whether male circumcision would reduce transmission of HIV and STIs from HIV-infected men to their uninfected female sexual partners. Here, we report the trial results from female partners of HIV-infected men, including frequency of HIV and rates of STI symptoms and vaginal infections.

Methods

Participants

The trial was done in Rakai District, Uganda, between 2003 and 2007. Trial procedures for HIV-infected men, including consent, randomisation, and data and sample collection, were the same as those previously reported in the trial of male circumcision in HIV-uninfected men.[3] In brief, men received an explanation of study goals and provided written informed consent for screening and HIV testing. Before screening and throughout the trial, men were offered HIV results, counselling, and information on HIV prevention. They were informed that the effects of male circumcision on transmission of HIV and STIs to female partners were unknown and that adherence to safe sexual practices was imperative.

Men aged 15–49 years were eligible for enrolment if they were HIV-infected, uncircumcised, had no medical indications or contraindications for circumcision, and, because the safety of male circumcision in HIV-infected men was unknown, had no evidence of immuno-suppression (WHO clinical stage 3 or 4, or a CD4-cell count below 350 cells per μL). Men with genital infections or a haemoglobin concentration of 80 g/L or less were treated and rescreened before enrolment.

Male participants in the trials of male circumcision in HIV-infected and HIV-uninfected men were asked to invite their wives or permanent consensual partners (hereafter referred to as female partners) to enrol in a study to assess the efficacy of male circumcision for the prevention of male-to-female HIV and STI transmission. Enrolment and follow-up procedures were the same for female partners irrespective of the male partner's HIV status. Results reported in this paper are for the partners of HIV-infected men.

Female partners were informed of study goals and procedures, told that the effects of male circumcision on transmission of HIV or STIs were unknown, and counselled on HIV and STI prevention (including consistent condom use) and on the need to refrain from sexual intercourse after their partner's circumcision until complete wound healing had been certified. All female participants provided written informed consent for enrolment and follow-up.

Randomisation and masking

Male participants were randomly assigned to receive circumcision within approximately 2 weeks of enrolment (intervention), or to have circumcision after 24 months (control). Treatment assignment was randomly generated in blocks of 20,[3] based on computer-generated random number sequences provided by the study statistician (LHM) at Johns Hopkins University (Baltimore, MD, USA), who undertook study analyses but had no contact with participants. Assignment sequences were placed in opaque, sealed envelopes and sent from John Hopkins University to Rakai in batches of 20. Trial enrolment and treatment assignment occurred in field hubs situated in several sites in Rakai District. At each hub, enrolment was carried out by trained Rakai Health Sciences Program clinical officers, who then asked each enrolled man to select a sealed envelope from a batch of 20. After an assignment envelope was selected, it was replaced by an envelope randomly selected from a second batch, to provide each participant with the opportunity to select from among 20 envelopes. Clinical officers who undertook enrolment and administered the randomisation process also undertook follow-up visits. In view of the surgical nature of the intervention, neither participants nor study clinicians could be masked to assignment group.

Procedures

Before circumcision, men were provided with detailed instructions on postoperative wound care, hygiene, abstention from sexual intercourse until complete wound healing had been certified, and safe sexual practices thereafter. They were given an information sheet with these instructions to share with their sexual partners. Circumcisions were done by use of the sleeve procedure.[3,5] Postoperative follow-up visits were scheduled at 24–48 h, 5–9 days, and 4–6 weeks, and predefined adverse events were recorded.[3,5] Men whose wounds were not fully healed at the 4–6-week visit were followed weekly until healing was certified.

At every postoperative follow-up visit, participants were interviewed and the wound was inspected. Participants were asked about resumption of sexual intercourse; those who resumed sex were asked when intercourse first occurred after surgery and whether condoms were used. The information about sexual risk reduction, including post-surgical sexual abstinence until complete wound healing, was reiterated at each postoperative visit. Male participants in both study groups were then followed at 6, 12, and 24 months post-enrolment, interviewed about sexual behaviours, health, and related issues, and examined. Venous blood samples and penile swabs were obtained.

Female partners of HIV-infected men were followed at 6, 12, and 24 months' post-enrolment. At baseline and every follow-up visit, women were given a detailed sociodemographic, behavioural, and health interview and provided venous blood samples and self-collected vaginal swabs. Interviews were done in private, by trained same-sex interviewers fluent in the Luganda language.

At every study visit, participating men and their female partners were provided with intensive education on HIV/STI prevention, including promotion of sexual abstinence, faithfulness, and consistent condom use. Additionally, both partners were offered free condoms, voluntary HIV counselling and testing, and couples' voluntary counselling and testing. Participants could be enrolled in the trial even if they declined to receive their HIV results or to disclose their results. Intensive efforts were made throughout the trial to facilitate individual and couples' counselling and disclosure of HIV results,

including the creation of couples' support clubs. Participants were informed of the advantages of receiving HIV results including, as of 2004, access to free antiretroviral therapy offered by the Rakai Health Sciences Program with funding from the President's Emergency Plan for AIDS Relief. In addition to the information provided to all participants, community meetings were undertaken to inform the population of the trial and of the need for safe sexual practices irrespective of the male partner's circumcision status.

The protocol was reviewed and approved by the Uganda National Council for Science and Technology, and by three institutional review boards: 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 (Baltimore, MD, USA), and the Western Institutional Review Board (Olympia, WA, USA). Trial oversight was provided by an independent data safety and monitoring board. A community advisory board provided guidance on study design, conduct, and the dissemination of results to the community. The trial was done in accordance with the Good Clinical Practice and International Clinical Harmonisation guidelines. Women were compensated for their time and travel costs, equivalent to US$3 per study visit, for a total of $12 for completion of all visits. The community advisory board, data safety and monitoring board, and institutional review boards approved this compensation as appropriate.

The parallel trial of male circumcision in HIV-uninfected men was closed on Dec 12, 2006, after an interim analysis showed the efficacy of male circumcision for HIV prevention in men.[3] Participants in both trials, as well as Rakai communities, were informed of this finding. Because continuation of the trial in HIV-infected men could result in stigmatisation of participants, enrolment of HIV-infected men was paused and the investigators requested an unscheduled interim review and guidance from the data safety and monitoring board. The board calculated that the conditional power to detect 60% efficacy, as specified in the study protocol, was only 4·9% and recommended that enrolment be closed. The investigators were unblinded, and study participants (men and women) were informed of the finding. However, the data safety and monitoring board recommended continued follow-up of enrolled participants. After a review of follow-up data on Dec 17, 2007, the data safety and monitoring board recommended that follow-up of HIV-infected men and their partners be closed. The current analysis is based on results up to that date.

The primary endpoint of the trial was the male-to-female HIV transmission rate. HIV status was assessed by two EIAs: Vironostika HIV-1 (Organon Teknika, Charlotte, NC, USA) and Welcozyme HIV 1+2 (Murex Diagnostics, Temple Hill, Dartford, UK). Discordant EIA
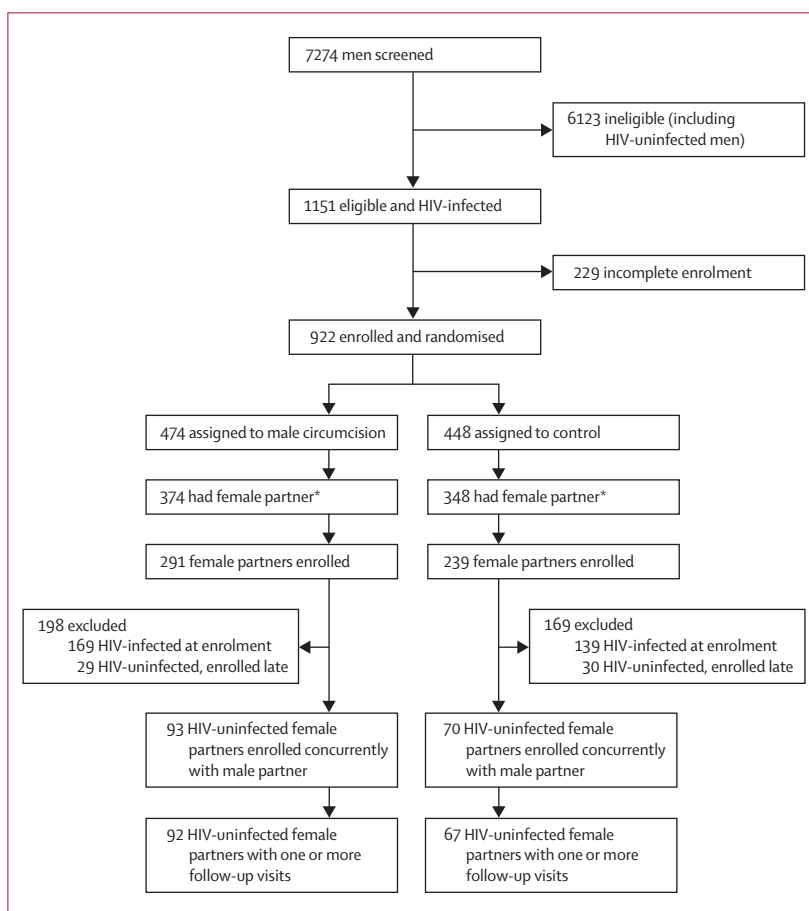
Figure 1: Trial profile
The intention-to-treat population included female partners of concurrently enrolled couples who had at least one follow-up visit over 24 months. *Female partner defined as wife or permanent consensual partner.

results and seroconversions were confirmed by western blot (Calypte Biomedical Corporation, Rockville, MD, USA). Male HIV viral load was measured by reverse transcriptase PCR assay (Amplicor HIV-1 Monitor version 1.5, Roche Molecular Systems, Branchburg, NJ, USA). Women's self-collected vaginal swabs were assessed for *Trichomonas vaginalis* by InPouch TV culture (BioMed Diagnostics, San Jose, CA, USA). Vaginal flora was quantified by the Nugent method;[10] a score of 7–10 was classified as bacterial vaginosis.

To ascertain whether each HIV-infected woman had acquired the virus from her linked partner, viral sequence data were generated from both individuals for portions of the *gag* and *gp41* fragments.[11] The genetic distance of the viral sequences between the two partners was compared with the variation between epidemiologically unrelated individuals in the Rakai population.[12,13]

Statistical analysis

On the basis of our previous observational data,[7] the study was powered to detect an incidence rate ratio of 0·41 for HIV transmission from HIV-infected men in the

	Men		Female partners	
	Intervention (n=87)	Control (n=68)	Intervention (n=93*)	Control (n=70*)
Enrolment age (years)				
15–19	0 (0%)	0 (0%)	4 (4%)	3 (4%)
20–29	25 (29%)	13 (19%)	60 (65%)	31 (44%)
30–49	62 (71%)	55 (81%)	29 (31%)	36 (51%)
Education				
None	1 (1%)	4 (6%)	13 (14%)	11 (16%)
Primary	69 (79%)	50 (74%)	74 (80%)	55 (79%)
Secondary or higher	17 (20%)	14 (21%)	6 (6%)	4 (6%)
Marital status				
Never married	..	..	0 (0%)	1 (1%)
Monogamous	74 (85%)	54 (79%)	76 (82%)	57 (81%)
Polygamous	13 (15%)	14 (21%)	17 (18%)	12 (17%)
Number of sexual partners in past year				
None	..	..	0 (0%)	2 (3%)
One	35 (40%)	26 (38%)	82 (88%)	63 (90%)
Two	35 (40%)	32 (47%)	9 (10%)	4 (6%)
Three or more	17 (20%)	10 (15%)	2 (2%)	1 (1%)
Condom use in past year				
None	47 (54)	27 (40%)	73 (78%)	44 (63%)
Inconsistent use	36 (41%)	35 (51%)	19 (20%)	22 (31%)
Consistent use	4 (5%)	6 (9%)	1 (1%)	4 (6%)
Alcohol use with sex in past year	63 (72%)	54 (79%)	35 (38%)	25 (36%)
Acceptance of HIV results at time of enrolment	85 (98%)	64 (94%)	64 (69%)	52 (74%)
Symptoms of STIs in past year				
Genital ulcer disease	23 (26%)	13 (19%)	15 (16%)	14 (20%)
Discharge/dysuria	18 (21%)	10 (15%)	47 (51%)	35 (50%)
Current symptoms of STI				
Genital ulcer disease	3 (3%)	0 (0%)	2 (2%)	1 (1%)
Discharge/dysuria	5 (6%)	3 (4%)	19 (20%)	15 (21%)
Syphilis serology†				
RPR negative/TPPA negative	74 (91%)	58 (88%)	77 (90%)	58 (85%)
RPR positive/TPPA negative	4 (5%)	1 (2%)	5 (6%)	4 (6%)
RPR positive /TPPA positive	3 (4%)	7 (11%)	4 (5%)	6 (9%)
Male partner HIV viral load				
Below detection (<400 copies per mL)	18 (21%)	6 (9%)	..	..
<50 000 copies per mL	47 (54%)	42 (62%)	..	..
≥50 000 copies per mL	22 (25%)	20 (29%)	..	..

RPR=rapid plasma reagin. TPPA=*Treponema pallidum* particle agglutination. STI=sexually transmitted infection. Data are n (%). *The number of men is less than the number of female partners enrolled because five men in the intervention group and two in the control group were polygamous and their co-wives were enrolled. In the analyses, correlation was accounted for by robust variance estimates. †Data available for 81 men and 86 female partners in the intervention group, and 66 men and 68 female partners in the control group.

Table 1: Enrolment characteristics of HIV-infected men and HIV-uninfected female partners in concurrently enrolled couples

surgery, or individuals in the control group who opted to have circumcisions done outside the study). On the basis of the previous observational data, we also postulated that transmission would be further reduced in couples in which the circumcised HIV-infected man had a viral load of less than 50 000 copies per mL and estimated that the study had more than 90% power to detect efficacy of 95% or more in this subgroup. No interim analyses were originally planned.

The research team undertook data analyses at Johns Hopkins University and at the Rakai Health Sciences Center. The effect of male circumcision on male-to-female HIV transmission was assessed in a modified intention-to-treat (ITT) analysis by use of Kaplan-Meier estimation, based on the time to the follow-up visit at which the female partner first tested HIV positive. The modified ITT population included all concurrently enrolled couples in which the female partner had at least one follow-up visit over 24 months. An overall risk difference and risk ratios were calculated at the end of follow-up, with 95% CIs based on Greenwood variance estimates. Cox proportional hazards models were used to estimate the adjusted hazard ratio (HR) of HIV detection in female partners, after adjustment for covariates that differed between study groups at enrolment by $p<0.15$ (differences in charac-teristics at enrolment were assessed by use of χ^2 tests). We assessed male-to-female HIV transmission by female-reported characteristics and behaviours at enrolment and follow-up. Female risk behaviours were also compared between assignment groups at each follow-up visit. We recorded the prevalence of vaginal infections and symptoms during follow-up, and estimated the prevalence risk ratios (PRRs) by use of modified Poisson regression with robust variance estimation to account for repeat observations.

After unblinding the study, the data safety and monitoring board requested further analyses, including male-to-female HIV transmission in couples in the intervention group by timing of resumption of sexual intercourse relative to certification of wound healing. In view of the schedule of postoperative visits, timing of healing could not be precisely established because healing preceded certification (ie, there was an unknown interval between actual and observed healing). We assumed that men who resumed sexual intercourse after certification of wound healing, or within the 5 days immediately before the study visit when certification occurred, were likely to have an intact scar; these men were classified as having delayed resumption of sex. Men who reported having resumed intercourse more than 5 days before the visit at which certification occurred, when scar formation was less likely to be complete, were classified as having early resumption of sex. We then assessed whether male-to-female HIV transmission had occurred by the 6-month follow-up visit in couples in the intervention group who reported early and delayed resumption of sex.

intervention group compared with the control group. We estimated that 220 couples would provide 80% power to detect this reduction over 2 years, adjusting for losses to follow-up and crossovers (ie, men who were originally allocated to circumcision but who did not present for

After the study was unblinded, we examined HIV viral load before and after male circumcision in consenting men from the control group who received circumcision as a service, to assess whether the stress of surgery might upregulate HIV viral load. 89 men who were not taking antiretroviral therapy and 25 men receiving antiretroviral therapy provided blood immediately before surgery and at the 1-month post-surgical visit. (During the trial, blood samples were not taken between the time of surgery and the 6-month follow-up visit.) We estimated within-individual change in $\log_{10}$ HIV viral load copies per mL after male circumcision, relative to the preoperative concentrations, by use of a paired t test. Stata version 8.0 was used for all analyses. This trial is registered with ClinicalTrials.gov, number NCT00124878.

Role of the funding source
FM and LHM had full access to all data until trial closure. All other investigators were masked until trial closure and had access to data thereafter. RR from the Gates Foundation maintained oversight of trial progress, and participated in open data safety and monitoring board sessions and in the interpretation of data. TCQ, SJR, and OL from the National Institute for Allergy and Infectious Diseases provided laboratory support and participated in the interpretation of data. The other sponsors 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 final responsibility for the decision to submit for publication.

Results
Figure 1 shows the trial profile. The modified ITT population for the analysis of male-to-female HIV transmission comprised 92 couples from the intervention group and 67 couples from the control group. An additional 29 HIV-uninfected female partners of men in the intervention group and 30 HIV-uninfected partners of men in the control group entered the study 6 months or more after their husband's enrolment. These women were excluded from the primary analysis because we did not know their HIV status at the time of their partner's enrolment. Therefore, we could not establish which women with delayed enrolment and HIV infection had seroconverted since their husband's enrolment, and this could result in bias if HIV transmission in the first 6 months differed by study group. The couples with delayed enrolment of female partners were assessed in secondary analyses.

Table 1 shows the characteristics of HIV-infected men and concurrently enrolled HIV-uninfected female partners at enrolment. Female partners in the intervention group were younger (p=0·067) and less likely to report condom use in the past year (p=0·017) than those in the control group. At enrolment, HIV results and post-test counselling were accepted by 85 (98%) men and 64 (69%)

	Intervention group	Control group
6-month visit	88/93 (95%)	63/70 (90%)
12-month visit	88/93 (95%)	65/70 (93%)
24-month visit*	49/60 (82%)	36/42 (86%)

Data are number of female participants seen at visit/number of female participants eligible at each visit (%). *The population at risk in the second year was diminished by losses to follow-up in the first year, and by women whose follow-up was truncated before 24 months because of early trial closure.

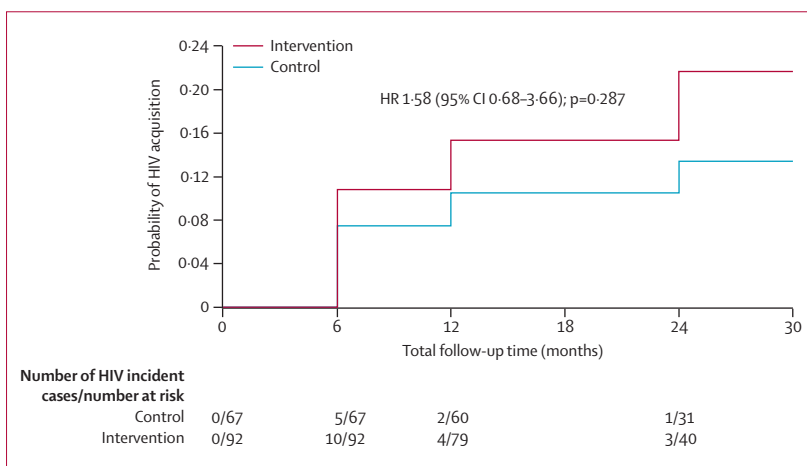*Table 2:* Female partner retention rates

Figure 2: Kaplan-Meier cumulative probability of female HIV acquisition by study group

female partners in the intervention group and by 64 (94%) men and 52 (74%) female partners in the control group. Since an additional 15 (16%) female partners in the intervention group and 11 (16%) in the control group reported that they had previously received their results, 79 (85%) and 63 (90%) women had received HIV results, respectively. All participants received intensive education on HIV prevention. Female retention rates were similar in both groups at the 6, 12, and 24-month follow-up visits (table 2).

17 (18%) women in the intervention group and eight (12%) women in the control group acquired HIV during follow-up (p=0·36). Figure 2 shows the Kaplan-Meier cumulative probabilities of female HIV acquisition in couples with concurrent male and female enrolment. Over the 24-month follow-up period, the cumulative probability of female acquisition of HIV was 21·7% (95% CI 12·7–33·4) in the intervention group and 13·4% (6·7–25·8%) in the control group (unadjusted HR 1·58, 95% CI 0·68–3·66; p=0·287). After adjustment for differences between groups in enrolment characteristics (ie, female enrolment age, female condom use in past year) by Cox proportional hazards regression, the adjusted HR was 1·49 (0·62–3·57; p=0·368). In a secondary analysis that included HIV-negative female partners who enrolled 6 months or more after their male partner, the cumulative probability of infection was 17·4% in the intervention group and 15·8% in the control group

	Number of female partners tested at 6 months†	Female HIV incident cases at 6-month follow-up	HIV acquisition (% [95% CI])	Intervention versus control		Early resumption of sex versus delayed resumption of sex in intervention group	
				Rate ratio (95% CI)	p value	Rate ratio (95% CI)	p value
Intervention group; early resumption of sex‡	18	5	27·8% (7·10–57·55)	3·50 (1·14–10·76)	0·038	2·92 (1·01–8·46)	0·060
Intervention group; delayed resumption of sex§	63	6¶	9·5% (3·26–15·74)	1·20 (0·39–3·73)	1·00	1·00	..
Control group	63	5	7·9% (1·26–14·61)	1·00	..	..	..

*Timing of resumption of sexual intercourse relative to certification of wound healing. †The denominators include women seen at 6 months for whom data was available on timing of resumption of sexual intercourse relative to their husband's wound healing. The total number of female partners from the intervention group with information on resumption of sex relative to their husband's wound healing (n=81), is lower than the 88 women seen at 6 months (table 2) because information on sexual resumption was obtained from the male partner. 77 (89%) men from the intervention group were followed up at 6 months. ‡Sex resumed more than 5 days before male partner's wound was certified as completely healed. §Sex resumed within the 5 days before or any time after certified wound healing. ¶One female partner missed the 6-month visit, was found to be HIV-infected at 12 months, and her husband reported delayed resumption of intercourse after surgery. It was conservatively assumed that infection occurred during the 0–6-month interval. If this infection actually occurred between 6 months and 12 months, HIV acquisition at the 6-month follow-up visit in women with delayed sexual resumption would be 7·9%, and the rate ratio of early versus late sexual resumption 3·44 (95% CI 1·12–10·59; p=0·04).

Table 3: HIV acquisition at 6 months in female partners in the control group and in the intervention group by timing of resumption of sexual intercourse*

(unadjusted HR 1·22, 95% CI 0·59–2·54; p=0·65). There were no male crossovers among couples included in the primary analysis. However, there were three crossovers among men in the intervention group whose female

	Intervention	Control	p value
Sexual behaviours*			
6-month visit			
Number of sexual partners			
None	2/88 (2%)	2/63 (3%)	0·89
One	83/88 (94%)	58/63 (92%)	..
Two or more	3/88 (3%)	3/63 (5%)	..
Condom use in sexually active participants			
None	55/86 (64%)	37/61 (61%)	0·16
Inconsistent use	8/86 (9%)	12/61 (20%)	..
Consistent use	23/86 (27%)	12/61 (20%)	..
Alcohol use with sex in sexually active participants	32/86 (37%)	24/61 (39%)	0·86
12-month visit			
Number of sexual partners			
None	3/88 (3%)	3/65 (5%)	0·87
One	76/88 (86%)	57/65 (88%)	..
Two or more	9/88 (10%)	5/65 (8%)	..
Condom use in sexually active participants			
None	47/85 (55%)	32/62 (52%)	0·36
Inconsistent use	8/85 (9%)	11/62 (18%)	..
Consistent use	30/85 (35%)	19/62 (31%)	..
Alcohol use with sex in sexually active participants	38/85 (45%)	27/62 (44%)	1·00
24-month visit			
Number of sexual partners			
None	3/49 (6%)	0/33 (0%)	0·48
One	42/49 (86%)	31/33 (94%)	..
Two or more	4/49 (8%)	2/33 (6%)	..
Condom use in sexually active participants			
None	21/46 (46%)	17/33 (52%)	0·28
Inconsistent use	2/46 (4%)	4/33 (12%)	..
Consistent use	23/46 (50%)	12/33 (36%)	..
Alcohol use with sex in sexually active participants	15/46 (33%)	12/33 (36%)	0·81
(Continues on next page)			

partner had delayed enrolment; none of these participants transmitted HIV to their partners.

In a subanalysis (not specified in the protocol), we assessed whether HIV transmission in couples in the intervention group was associated with the timing of resumption of sexual intercourse relative to wound healing (table 3). Female acquisition of HIV, assessed at 6 months, occurred in a higher proportion of couples who resumed sex early than in couples who delayed resumption of sex. The rate of female acquisition of HIV in couples from the control group was similar to that for couples in the intervention group who delayed resumption of sex but significantly lower than the rate in couples who resumed sex early.

There were no significant differences in HIV transmission between study groups by enrolment covariates, or by female-reported sexual risk behaviours during follow-up (data not shown). In women whose partner's viral load was less than 50 000 copies per mL at enrolment, the cumulative probability of HIV acquisition was 17% (11 of 65) in the intervention group and 10% (five of 48) in the control group (HR 1·48, 0·55–3·98; p=0·43). The corresponding values for women whose partner's viral load was more than 50 000 copies per mL were 27% (six of 22) and 15% (three of 20), respectively (HR 1·82, 0·52–6·32; p=0·34).

Number of sexual partners, condom use, and use of alcohol with sex reported by female partners during follow-up did not differ between groups (table 4). 70 (75%) of 93 HIV-infected men in the intervention group disclosed their serostatus to their female partner compared with 54 (77%) of 70 controls (p=0·38).

The proportions of follow-up visits at which female partners reported symptoms of STIs or had laboratory-diagnosed bacterial vaginosis did not differ between groups (table 4). The prevalence of trichomonas infection was lower in female partners in the intervention group than in the control group (PRR 0·43, 95% CI 0·18–1·02; p=0·056).

Sequence data for both partners were available for 13 of 25 couples in which the female partner seroconverted during the trial. In all 13 couples, the genetic distance of the viral sequences between partners was less than 0·5%, which was less than two SDs below the median distance of sequences between unrelated individuals in Rakai, indicating probable HIV acquisition within the partnership.[13]

We assessed preoperative and postoperative HIV viral load in 89 men from the control group who had never received antiretroviral therapy and who received male circumcision as a service after trial closure. In 80 men with detectable viral load before surgery, mean viral load was 4·30 $\log_{10}$ copies per mL (SD 0·83) preoperatively, and 4·50 $\log_{10}$ copies per mL (0·74) 4 weeks after surgery, a mean increase in intra-individual viral load of 0·20 $\log_{10}$ copies per mL (p=0·002). All nine men with undetectable viral load before surgery remained undetectable at week 4. In an analysis of 25 men from the control group who had started antiretroviral therapy before circumcision, we found no increase in viral load in 21 (84%) men who had an undetectable viral load before surgery, or in four men who had a detectable preoperative viral load.

Surgery-related adverse events of male circumcision recorded in this trial are reported elsewhere.[3,5]

Discussion

In this trial, circumcision of HIV-infected men did not reduce transmission of the virus to uninfected female partners. Furthermore, we cannot exclude the possibility of higher HIV transmission in couples who resumed intercourse before complete healing of the surgical wound. Since the duration of the study was limited and not all female partners completed 24 months of follow-up, we could not assess the long-term benefits or risks of male circumcision for HIV transmission to women. The findings suggest that strict adherence to sexual abstinence during wound healing and consistent condom use thereafter must be strongly promoted when HIV-infected men receive circumcision.

These findings have important implications for male circumcision programmes. WHO and UNAIDS recommend that surgery should not be denied to HIV-infected men who request the procedure unless there are medical contraindications.[4] Despite the absence of efficacy of male circumcision for HIV prevention in women, we agree with these recommendations for several reasons. First, if programmes excluded HIV-infected men it could result in stigmatisation, and HIV-infected men might seek surgery from potentially unsafe sources to mask their serostatus. Second, circumcised HIV-uninfected men might use their circumcised status to negotiate unsafe sex. Finally, circumcision reduces genital ulcer disease[6] and human papillomavirus infection in HIV-infected men (Gray RH, unpublished), which suggests direct health benefits of the intervention.

	Intervention	Control	p value
(Continued from previous page)			
STIs†			
Reported symptoms of STIs			
Genital ulcer disease	37/225 (16%)	26/161 (16%)	0·95
Vaginal discharge	82/225 (36%)	52/161 (32%)	0·50
Dysuria	35/225 (16%)	24/161 (15%)	0·89
Laboratory-diagnosed infections			
Bacterial vaginosis	121/217 (56%)	83/160 (52%)	0·54
Trichomonas vaginalis	9/138 (7%)	17/112 (15%)	0·056

STIs=sexually transmitted infections.*Data are n/N (%). Denominators for number of sexual partners at each visit are for all respondents seen at the visit; denominators for condom and alcohol use are based on women who reported being sexually active since the previous visit. †Data are number of events reported to have occurred between visits/number of follow-up visits.

Table 4: **Sexual behaviours and sexually transmitted infections in female partners during follow-up, by study group**

The association between resumption of sexual intercourse before complete wound healing and increased risk of male-to-female HIV transmission makes it imperative that circumcised men and their female partners are clearly instructed to abstain from intercourse until the wound is healed. We previously reported that wound healing was complete in 73% of HIV-infected men at 4 weeks and in 93% at 6 weeks after male circumcision.[5] Thus, it would be prudent to recommend sexual abstinence for a minimum of 6 weeks after surgery. At 6 weeks, men should be re-examined to assess healing before resumption of sexual intercourse is advised. The possible short-term increase in HIV transmission to partners of circumcised HIV-infected men if sex is resumed early is unlikely to have a substantial effect on the HIV epidemic: the exposure period of possible increased risk is short and the number of HIV-infected men with uninfected female partners who resume sex early will generally represent a small proportion of recipients of male circumcision. Nonetheless, comprehensive male circumcision programmes should, wherever feasible, promote and offer condoms, voluntary HIV counselling and testing, couples' counselling and testing, and health messages for women about male circumcision. Circumcising infants and boys before their sexual debut would mitigate the challenges of male circumcision in HIV-infected men; however, this strategy would require careful consideration of issues related to parental consent and the minor's assent.

We recorded an increase in HIV viral load in antiretroviral-naive men after surgery, which could result in higher infectivity.[14] Our post-surgical assessment was undertaken at 4 weeks, and additional research is needed to establish whether male circumcision affects viral load beyond this period.

We were disappointed that the trial did not show protection from HIV infection in women, as was expected from observational studies.[7–9] One possible explanation is

that most men in the observational studies had been circumcised in childhood and did not initiate intercourse until long after complete wound healing. However, it should be noted that we did not find a difference in HIV incidence rates between women from the intervention group and women from the control group at 12 months and 24 months, substantial periods of time after wound healing appeared complete.

We previously reported that female partners of HIV-uninfected men assigned to male circumcision had lower rates of genital ulcer disease, trichomonas infection, and bacterial vaginosis than did controls.[15] In partners of HIV-infected men, male circumcision was associated with lower rates of trichomonas infection (PRR 0·43, 95% CI 0·18–1·02; p=0·056), similar to the PRR in partners of HIV-uninfected men.[15] However, male circumcision in HIV-infected men was not associated with reduced rates of STI symptoms or bacterial vaginosis in female partners.

There are some limitations to this study. The trial was underpowered, in part because the number of concurrently enrolled HIV-discordant couples with HIV-infected men was lower than specified by the protocol. Although the proportions of married men were similar in both study groups, a higher proportion of female partners in the intervention group were enrolled in the study (78%) than were partners in the control group (69%; p=0·007). This finding suggests differential motivation to participate between trial groups, which could have introduced bias. However, apart from younger age and lower condom use reported by women in the intervention group than by women in the control group, female baseline characteristics did not differ between groups and adjustment for the differences did not materially affect the estimates of efficacy. The study was closed early and this restricted our ability to assess longer-term effects of male circumcision. 29 female partners in the intervention group and 30 in the control group were enrolled 6 months or more after their male partners, and were excluded from the primary analysis; however, inclusion of these late enrollees in secondary analyses did not alter the results. Finally, for reasons of safety, we excluded HIV-infected men with CD4-cell counts less than 350 cells per μL or with WHO clinical stage 3 or 4 disease. Thus, the current study cannot establish possible effects on female partners of circumcision in men with more advanced HIV infection.

Most trials in HIV-discordant couples enrol male and female participants as couples. However, in this trial HIV-infected men were enrolled and randomised as individuals, and then asked to invite their partners, who also enrolled as individuals. Participants were strongly encouraged to accept couples' voluntary counselling and testing at enrolment and throughout the trial, but about a quarter in each group did not disclose their serostatus. In our previous experience, acceptance of couples' voluntary counselling and testing within marital and long-term consensual couples is fairly low in this rural population and is more frequent if both partners know that they are not infected with HIV. HIV transmission rates in this study, particularly in the first 6 months, were high compared with studies of HIV-discordant couples enrolled after receipt of couples' counselling and testing. The latter couples might represent a self-selected and motivated subpopulation and might be more likely to adopt preventive behaviours[16] than the individuals in this trial. For example, consistent condom use was uncommon at enrolment, increased over time, but was still quite low at 24 months (50% in the intervention group and 36% in the control group), despite repeated health education and the provision of free supplies. Inclusion of only couples who agreed before enrolment to couples' counselling and result disclosure might have resulted in lower HIV transmission rates in both trial groups, including potentially reduced early postoperative HIV transmissions, since it is possible that these couples might have been more compliant with the recommendations to delay intercourse after circumcision surgery and to use condoms consistently thereafter.

It would be difficult to undertake another trial of effects of male circumcision on male-to-female HIV transmission. In view of our results, such a trial would have to be powered to detect a low efficacy, which would require a very large population of male-infected HIV-discordant couples, as well as protracted follow-up. Since higher transmission rates in the post-surgical period are a possibility, additional follow-up visits and interim safety analyses would be needed. Therefore, costs, logistics, and limited expectation of efficacy probably render such a trial unfeasible.

Thus, circumcision of HIV-infected men did not reduce transmission of HIV to female partners, and the possibility of higher risk of transmission in couples who resumed sexual intercourse before complete wound healing cannot be excluded. Wherever possible, male circumcision should be offered in conjunction with HIV counselling services, condoms, and education on HIV prevention for men and women, to improve the health and safety of circumcised patients and their partners. However, the efficacy of male circumcision for prevention of HIV in uninfected men is clear,[1–3] and reductions in male acquisition of HIV attributable to circumcision are likely to reduce women's exposure to HIV-infected men.[17,18] Male circumcision programmes are thus likely to confer an overall benefit to women.

References
1 Auvert B, Taljaard D, Lagarde E, Sobngwi-Tambekou J, Sitta M, Puren A. Randomized, controlled intervention trial of male circumcision for reduction of HIV infection risk: The ANRS 1265 trial. *PLoS Med* 2005; **2:** 1112–22.
2 Bailey RC, Moses S, Parker CB, et al. Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial. *Lancet* 2007; **369:** 643–56.
3 Gray RH, Kigozi G, Serwadda D, et al. Male circumcision for HIV prevention in men in Rakai, Uganda: a randomised trial. *Lancet* 2007; **369:** 657–66.
4 WHO/UNAIDS. New data on male circumcision and HIV prevention: policy and programme implications. 2007. http://data.unaids.org/pub/Report/2007/mc_recommendations_en.pdf (accessed May 28, 2009).
5 Kigozi G, Gray RH, Wawer MJ, et al. The safety of adult male circumcision in HIV-infected and uninfected men in Rakai, Uganda. *PLoS Med* 2008; **5:** e116.
6 Wawer MJ, Kigozi G, Serwadda D, et al. Trial of male circumcision in HIV+men and in women partners. 15th Conference on Retroviruses and Opportunistic Infections; Boston, MA, USA; Feb 3–6, 2008. Abstract 33lb.
7 Gray RH, Kiwanuka N, Quinn TC, et al. Male circumcision and HIV acquisition and transmission: cohort studies in Rakai, Uganda. Rakai Project Team. *AIDS* 2000; **14:** 2371–81.
8 Kapiga SH, Lyamuya EF, Lwihula GK, Hunter DJ. The incidence of HIV infection among women using family planning methods in Dar es Salaam, Tanzania. *AIDS* 1998; **12:** 75–84.
9 Hunter DJ, Maggwa BN, Mati JK, Tukei PM, Mbugua S. Sexual behavior, sexually transmitted diseases, male circumcision and risk of HIV infection among women in Nairobi, Kenya. *AIDS* 1994; **8:** 93–99.
10 Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol* 1991; **29:** 297–301.
11 Yang C, Dash B, Hanna SL, et al. Predominance of HIV type 1 subtype G among commercial sex workers from Kinshasa, Democratic Republic of Congo. *AIDS Res Hum Retroviruses* 2001; **17:** 361–65.
12 Trask SA, Derdeyn CA, Fideli U, et al. Molecular epidemiology of human immunodeficiency virus type 1 transmission in a heterosexual cohort of discordant couples in Zambia. *J Virol* 2002; **76:** 397–405.
13 Wawer MJ, Gray RH, Serwadda D, et al. HIV transmission per coital act by stage of index partner infection in discordant couples, Rakai Uganda. *J Infect Dis* 2005; **191:** 1403–409.
14 Quinn TC, Wawer MJ, Sewankambo N, et al. HIV serum viral load, risk factors and HIV transmission in discordant couples, Rakai, Uganda. *N Engl J Med* 2000, **342:** 921–29.
15 Gray RH, Kigozi G, Serwadda D, et al. The effects of male circumcision on female partners' genital tract symptoms and vaginal infections in a randomized trial in Rakai, Uganda. *Am J Obstet Gynecol* 2008; **200:** 42e1–7.
16 Lingappa JR, Kahle E, Mugo N, et al. Characteristics of HIV-1 discordant couples enrolled in a trial of HSV-2 suppression to reduce HIV-1 transmission: the partners study. *PLoS One* 2009; **4:** e5272.
17 Hallett TB, Singh K, Smith JA, White AG, Abu-Raddad LJ, Garnett GP. Understanding the impact of male circumcision interventions on the spread of HIV in southern Africa. *PLoS One* 2008; **3:** e2212.
18 White RG, Orroth KK, Freeman EE, et al. Male circumcision for HIV prevention in sub-Saharan Africa: who, what and when? *AIDS* 2008; **22:** 1841–50.