Male circumcision and HIV acquisition and transmission: cohort studies in Rakai, Uganda

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Background: Male circumcision is associated with reduced HIV acquisition.

Methods: HIV acquisition was determined in a cohort of 5507 HIV-negative Ugandan men, and in 187 HIV-negative men in discordant relationships. Transmission was determined in 223 HIV-positive men with HIV-negative partners. HIV incidence per 100 person years (py) and adjusted rate ratios (RR) and 95% confidence intervals (CI) were estimated by Poisson regression. HIV-1 serum viral load was determined for the seropositive partners in HIV-discordant couples.

Results: The prevalence of circumcision was 16.5% for all men; 99.1% in Muslims and 3.7% in non-Muslims. Circumcision was significantly associated with reduced HIV acquisition in the cohort as a whole (RR 0.53, CI 0.33–0.87), but not among non-Muslim men. Prepubertal circumcision significantly reduced HIV acquisition (RR 0.49, CI 0.26–0.82), but postpubertal circumcision did not. In discordant couples with HIV-negative men, no serconversions occurred in 50 circumcised men, whereas HIV acquisition was 16.7 per 100 py in uncircumcised men ($P = 0.004$). In couples with HIV-positive men, HIV transmission was significantly reduced in circumcised men with HIV viral loads less than 50 000 copies/ml ($P = 0.02$).

Interpretation: Prepubertal circumcision may reduce male HIV acquisition in a general population, but the protective effects are confounded by cultural and behavioral factors in Muslims. In discordant couples, circumcision reduces HIV acquisition and transmission. The assessment of circumcision for HIV prevention is complex and requires randomized trials.

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Introduction

Prospective studies have shown increased acquisition of HIV infection in uncircumcised compared with circumcised men in selected high-risk populations, such as clients of commercial sex workers, men attending sexually transmitted disease (STD) clinics and Kenyan transport employees [1–6]. Also, in a study of discordant couples, we found lower levels of HIV acquisition in circumcised HIV-negative men [7]. However, no prospective studies have been conducted in representative general populations. Cross-sectional and ecological studies also suggest that circumcision may protect men from prevalent HIV and STD infections [8,9], and the protective effects of circumcision are most marked if the procedure is performed before the onset of puberty [10]. On the basis of these findings from observational studies, it has been proposed that circumcision should be widely promoted as a means of HIV prevention [8,9]. To assess the role of male circumcision in HIV prevention, we examined the effects of circumcision on HIV acquisition in a representative population-based cohort of Ugandan men with moderate potential HIV exposure, and we assessed both HIV acquisition and transmission in a group of HIV-discordant couples with high levels of HIV exposure.

Methods

The Rakai STD Control for AIDS Prevention Study was a community-randomized trial conducted in a rural area of southwestern Uganda. The methods and results have been reported previously [11,12]. All consenting adults aged 15–59 years resident in 10 community clusters were enrolled and followed at intervals of 10 months between November 1994 and October 1998. The follow-up rates were approximately 75%. The 10 clusters were randomly allocated five to an intervention and five to a control arm. The five intervention arm clusters received antibiotic treatment for STD control using a mass treatment strategy (i.e. treatment of all consenting symptomatic and asymptomatic subjects), and the five control arm clusters received mass treatment with anthelminth and vitamin supplements. Subjects were interviewed in the home to determine sociodemographic, behavioral and health-related characteristics. Men were asked whether they had been circumcised and, if so, at what age the procedure was performed. A venous blood sample was obtained for HIV testing using two enzyme immunoassays (EIA; Vironostika HIV, Organon Teknika, Charlotte, NC, USA and Cambridge Biotech, Worcester, MA, USA), with Western blot confirmation of discordant EIA tests (HIV WB; Bio-Merieux-Vitek, St Louis, MO, USA). Among HIV-positive subjects in discordant relationships with a HIV-negative partner, HIV-1 RNA in sera was quantified by reverse transcriptase polymerase chain reaction assay using the Amplicor HIV-1 Monitor 1.5 Assay (Roche Molecular Systems, Branchburg, NJ, USA). The minimal detectable range of HIV-1 RNA was 400 copies/ml. Urine was also tested for HIV using EIA (Calypte HIV Urine EIA; Calypte Biomedical, Alameda, CA, USA) with Western blot confirmation, for 10% of subjects who declined to provide a blood sample. Syphilis serology used the non-treponemal toluidine red unheated serum test (New Horizons, Columbia, MD, USA) with confirmation by *Treponema pallidum* hemagglutination test (Sero-Tek, Fujirebio, Tokyo, Japan). Gonorrhea and chlamydia infections were determined in a subsample of 2440 men using ligase chain reaction on first-catch urine samples (LCx Probe System; Abbott Laboratories, Abbott Park, IL, USA).

The association between circumcision and HIV incidence was examined in 5507 initially HIV-negative men, observed for 10 231 person years (py) in the whole cohort population. Individuals who were married or in stable relationships were asked to identify their partners. In the cohort of 5507 HIV-negative men, 3010 reported that they were currently married or in a consensual union, and linked data on the female partner's HIV status was available for 2732 couples (90.8%), of whom 2553 were concordant HIV-negative couples. There were 410 HIV discordant (HIV+ HIV−) couples in which the male partner's circumcision status was known. We assessed HIV acquisition associated with male circumcision in 187 HIV-negative men in discordant relationships with an HIV-positive female partner, and we examined HIV transmission by 223 HIV-positive men in discordant relationships with HIV-negative female partners. All couples were identified retrospectively in 1999, and linked data on the serostatus of partners were not available during the conduct of the trial (1994–1998).

All subjects were strongly encouraged to receive their HIV results and post-test counseling was provided at no cost by trained project counselors in confidence and privacy. Intensive efforts were made to provide HIV results to all participants without stigmatization, and approximately 60% of subjects requested and received their HIV test results and counseling during the course of the trial. Ugandan Ministry of Health policy en-

courages voluntary HIV testing/counseling and the sharing of results between sexual partners, but does not allow involuntary provision of HIV test results to partners within HIV-discordant relationships. All subjects also received health education and condom promotion, and condoms were provided free of charge by the project. The study was approved by Institutional Review Boards in Uganda, Columbia and Johns Hopkins universities, and the National Institutes of Health.

The characteristics of circumcised and uncircumcised men were compared, and differences assessed by χ^2 tests. Incidence rates of HIV seroconversion were estimated per 100 py, and 95% confidence intervals (CI) were estimated from the standard errors of these rates. Tests of statistical significance for differences in HIV incidence associated with circumcision were based on comparison of the CI of the incidence rates, and on estimation of the rate ratio (RR) and 95% CI of HIV acquisition rates in circumcised men compared with the incidence in uncircumcised men. Stratified analyses were conducted to assess possible confounding or interaction. Circumcision is highly correlated with Islamic religion in this population, so to determine whether religious affiliation affected the risk of HIV, we examined HIV acquisition associated with religion (Muslim/non-Muslim) in separate stratified analyses restricted to circumcised men. Also, we previously found that age at circumcision was associated with prevalent HIV infection [10], so analyses were stratified by circumcision at or before 12 years of age (the approximate age of onset of male puberty in this population), versus procedures performed at older ages. Multivariate adjusted risks of HIV acquisition associated with circumcision were estimated using Poisson regression models [13], incorporating covariates for age (15–19, 20–29, 30–39, 40+ years), marital status (never married, monogamous, polygamous, previously married), number of reported extramarital sex partners in the past year (none, 1+) and STD diagnosis. Adjustment was also made for trial randomization arm. Because circumcision was almost universal among Muslim men, a variable for religion could not be included in the main regression models. However, separate stratified models were fitted for non-Muslim men among whom the HIV risks associated with circumcision and age at circumcision could be determined, without confounding by religious affiliation.

The analyses of the discordant couples were as follows. HIV acquisition per 100 py was determined in 187 HIV-negative men with HIV-infected female partners, and transmission rates per 100 py were estimated in 223 couples with HIV-positive men in discordant relationships with HIV-negative female partners. Acquisition and transmission rates were also estimated in relation to the HIV viral load of the HIV-positive partner, stratified into viral loads of less than 10 000, 10 000–49 000 and 50 000 plus copies/ml. Multivariate adjusted risks of HIV acquisition or transmission associated with circumcision were estimated by Poisson regression, after adjustment for viral load of the HIV-positive index partner and the other covariates listed above.

Results

In the cohort of 5507 HIV-negative men, 908 men reported circumcision (prevalence 16.5%). Reasons given for circumcision were traditional/religious (87.0%), health (11.0%), or other reasons (2.0%). Table 1 compares the characteristics, sexual behaviors, STD symptoms and diagnoses in circumcised and uncircumcised men in the cohort. Compared with the uncircumcised men, the circumcised men were predominantly Muslim, significantly older, less frequently single and more often polygamously married. There were no differences between circumcised and uncircumcised men with respect to educational attainment, the number of extramarital sexual partners reported in the previous year or current condom use, but alcohol consumption within the past month was significantly less common in the circumcised than in the uncircumcised men, consistent with the predominance of Muslims among the circumcised. There was also a higher frequency of dysuria reported by circumcised men, but no significant differences were observed in other STD symptomatology, condom use or STD diagnoses. The proportions of men who were circumcised in the intervention arm were lower than in the control arm, because, by chance, the latter randomization group contained more Islamic communities [11,12].

Table 2 shows the incidence of HIV per 100 py among circumcised and uncircumcised men. HIV incidence was lower in the circumcised (1.1 per 100 py), compared with the uncircumcised men (1.8 per 100 py), and this difference was statistically significant (unadjusted RR = 0.61, CI 0.37–0.97). Among men who reported circumcision at or before the age of 12 years, the incidence of HIV was 0.9 per 100 py; the incidence of HIV was 1.5 per 100 py in men reporting circumcision at 13 years or older, and incidence was 1.8 per 100 py in the uncircumcised (χ^2 for trend 4.97, $P = 0.03$). The HIV incidence in men with prepubertal circumcision was significantly lower than the HIV incidence of 1.8 per 100 py in the uncircumcised men (unadjusted RR = 0.54, CI 0.40–0.71), but for men circumcised at 13 years or older, HIV incidence was not significantly different from the incidence in the uncircumcised men (unadjusted RR = 0.83, CI 0.35–2.03).

Table 1. Population cohort of 5507 HIV-negative men: characteristics, behaviors, sexually transmitted disease symptoms and diagnoses in circumcised and uncircumcised men.

Characteristics, behaviors, STD symptoms and STD diagnoses	Circumcised (N = 908)		Uncircumcised (N = 4599)	
	No.	%	No.	%
Age (years)				
15–19	195	21.5	1252	27.2
20–29	317	34.9	1735	37.7
30–39	210	23.1	748	16.3
40–49	108	11.9	454	9.9
50–	78	8.6	410	8.9***
Marital status				
Never married	281	31.0	1900	41.3
Married monogamous	419	46.2	2090	45.4
Married polygamous	165	18.2	336	7.3
Divorced/separated/widowed	43	4.7	270	5.9***
Religion				
Muslim	730	80.4	7	0.2***
Other religions	178	19.6	4592	99.8***
Education				
No education	57	6.3	299	6.5
Primary education	583	54.3	3012	65.5
Secondary or higher education	267	29.4	1287	28.0
Sexual behaviors				
No extramarital partners in past year	591	65.1	3048	66.3
1– Extramarital partners in past year	309	34.0	1550	33.9
Current condom use	97	10.7	532	11.6
Alcohol use in past month	150	16.5	2982	64.8***
STD				
Genital ulcer in past year	65	7.2	297	6.5
Genital discharge in past year	33	3.6	156	3.4
Dysuria in past year	92	10.1	351	7.6*
Positive syphilis serology	93	10.2	482	10.5
Gonorrhea[a]	3	0.7	25	1.1
Chlamydia[a]	17	3.9	71	3.2
Randomization arm				
Intervention	421	46.4	2507	54.5***
Control	487	53.4	2103	45.7***

χ^2 test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.
[a]Subsample based on 438 circumcised and 2202 uncircumcised men with urinary ligase chain reaction results.
STD, Sexually transmitted diseases.

HIV incidence was similar in circumcised and uncircumcised adolescent males aged 15–19 years. However, in men over the age of 20 years, HIV seroconversion rates were lower in the circumcised than the uncircumcised, although the differences were not statistically significant within age strata. A total of 535 men over the age of 20 years reported prepubertal circumcision, and HIV incidence was 1.0 per 100 py (10/1042 py), in 172 who reported circumcision at or above 13 years the HIV incidence was 1.5 per 100 py (5/325 py), and among 3351 uncircumcised men the incidence was 2.1 per 100 py (134/6414 py). This trend in HIV incidence by the age of circumcision or lack of circumcision was statistically significant ($\chi^2 = 6.2$, $P = 0.01$). Circumcision was not associated with a reduced risk of HIV acquisition in the never-married men (HIV incidence of 1.6 and 1.4 per 100 py in circumcised and uncircumcised men, respectively). However, among ever-married men, the rate of HIV acquisition was lower in the circumcised (0.9 per 100 py) than in the uncircumcised (2.1 per 100 py), and this difference was statistically significant (Table 2). Only seven Muslim men reported that they were uncircumcised, and none seroconverted. HIV incidence was similar in circumcised and uncircumcised non-Muslim men (1.6 and 1.8 per 100 py, respectively, Table 2). However, HIV acquisition was significantly lower in circumcised compared with uncircumcised men reporting no extramarital sexual partners (unadjusted RR = 0.50, CI 0.24–0.95) and those reporting no alcohol consumption within the previous month (unadjusted RR = 0.47, CI 0.26–0.91). There were no significant protective effects of circumcision on HIV acquisition in men reporting extramarital sexual partners, the use of condoms, alcohol consumption, a history of STD symptoms, diagnosed STD or by randomization arm.

Table 3 shows the multivariate Poisson regression used to estimate the adjusted rate ratio of HIV acquisition associated with circumcision. In the whole population, the adjusted rate ratio of HIV acquisition associated

Table 2. HIV incidence rates by circumcision status and selected sociodemographic/behavioral and health characteristics.

	Circumcised HIV-negative men			Uncircumcised HIV-negative men		
	No.	Incident HIV cases/py	HIV incidence/ 100 py	No.	Incident HIV cases/py	HIV incidence/ 100 py
All	908	18/1683	1.1	4608	154/8548	1.8**
Age at circumcision (years)						
≤ 12	726	13/1348	0.9	na		
13–	178	5/335	1.5	na		
Age (years)						
15–19	195	3/313	1.0	1252	20/2120	0.9
20–29	317	8/583	1.4	1735	69/3156	2.2
30–39	210	5/423	1.2	748	33/1459	2.3
40–	186	2/372	0.5	864	32/1792	1.8
Marital status						
Never married	281	7/452	1.6	1900	44/3248	1.4
Ever married	627	11/1239	0.9	2696	110/5274	2.1**
Religion						
Muslim	730	13/1373	1.0	7	0/16	0
Other religion	178	5/318	1.6	4592	154/8512	1.8
Sexual behaviors						
No extramarital partners	591	9/1132	0.8	3048	94/5763	1.6*
1– extramarital partners	240	6/434	1.4	1558	41/1704	2.4
Current condom use	97	1/175	0.6	450	16/798	2.0
No current condom use	810	17/1514	1.1	4145	138/7721	1.8
Alcohol past month	150	6/268	2.2	2982	104/5678	1.8
No alcohol use past month	752	12/1412	0.8	1628	50/2872	1.7*
STD						
GUD	65	2/113	1.8	297	11/575	1.9
Dysuria	92	3/174	1.7	351	11/695	1.6
Discharge	33	0/59	0	156	8/315	2.5
Syphilis	93	5/183	2.7	482	30/968	3.1
Gonorrhea	3	0/7	0	25	2/45	4.5
Chlamydia	17	2/29	6.8	71	5/139	3.6
Randomization arm						
Intervention	415	7/778	0.9	2507	92/4696	2.0
Control	487	11/902	1.2	2103	62/3854	1.6

χ^2 test on difference in HIV incidence among circumcised and uncircumcised men: * $P < 0.05$; ** $P < 0.01$.
GUD. Genital ulcer disease; py, person years; STD, sexually transmitted diseases.

Table 3. Adjusted rate ratios of HIV acquisition based on multivariate Poisson regression.

Covariates	Adjusted rate ratio of HIV incidence	95% confidence intervals
Any circumcision	0.53	0.33–0.87
Circumcision at ≤12 years	0.49	0.26–0.82
Circumcision at age 13–	0.70	0.25–1.55
Age (years)		
15–19	1.0	
20–29	2.02	1.19–3.46
30–39	1.92	1.01–3.37
40–59	1.38	0.71–2.70
Marital status		
Never married	1.0	
Married monogamous	0.89	0.58–1.38
Married polygamous	1.34	0.72–2.48
Separated divorced widowed	1.10	0.52–2.31
Sex partners in past year		
One	1.0	
Two	1.28	0.84–1.96
Three or more	1.75	1.10–2.79
Sex for money or gifts	1.05	0.76–1.47
Condom use	1.15	0.76–1.71
Syphilis	1.64	1.04–2.59

with circumcision was 0.53 (CI 0.33–0.87). For circumcision performed at 12 years or younger, the rate ratio was 0.49 (CI 0.26–0.82), and for circumcision performed at 13 year or above, the rate ratio was 0.70 (CI 0.25–1.55). Other covariates significantly associated with HIV risk were age (20–29 and 30–39 years), more than three sex partners in the previous year and positive syphilis serology. In separate models for non-Muslim men, the adjusted rate ratio of HIV acquisition associated with circumcision was 0.80 (CI 0.33–1.95) and was not statistically significant. Among non-Muslim men, circumcision at or before 12 years was associated with an adjusted rate ratio of HIV acquisition of 0.71 (CI 0.18–2.85), and for circumcision at or after 13 years, the rate ratio was 0.89 (CI 0.28–2.82).

Effects of religion on HIV acquisition among circumcised men

Religion was highly correlated with circumcision status. In Muslim men, 99.1% were circumcised (730/

737), and among the 730 circumcised Muslims, 87.5% reported circumcision at or before 12 years of age. All Muslims reported that circumcision was performed for religious reasons. However, in men of non-Muslim religious affiliations, the prevalence of circumcision was 3.7% (178/4770); 48.0% of those circumcised reported prepubertal circumcision and 52.0% were circumcised after 12 years of age. Moreover, 75% of the non-Muslim men with post-pubertal circumcision indicated that the procedure was performed for health reasons. The associations between circumcision and HIV acquisition shown in Table 2 may thus reflect behavioral differences between Muslim and non-Muslim men, rather than an effect of circumcision *per se*.

The potential effects of religion on HIV incidence cannot be assessed among the uncircumcised men, because there were too few uncircumcised Muslim men for meaningful analyses. Therefore, we compared HIV incidence in circumcised Muslim and non-Muslim men to assess the effects of religion, after controlling for circumcision status (Table 4). HIV incidence was 1.0 per 100 py in Muslim men and 1.6 per 100 py in circumcised non-Muslims, but this was not statistically significant (RR = 0.63; CI 0.21–2.07). Also, circumcised Muslims had a lower HIV incidence than circumcised non-Muslims, irrespective of the age at which circumcision was performed, although these differences were not statistically significant. There were no significant differences in HIV acquisition between Muslims and non-Muslims in the youngest and oldest age groups, but among men aged 20–29 years, HIV incidence was significantly lower in the Muslim men, compared with the non-Muslims (1.0 and 4.1 per 100 py, respectively, RR = 0.24, CI 0.05–0.77). The lower HIV incidence among circumcised Muslim compared with non-Muslim men aged 20–29 years was observed both in men reporting prepubertal circumcision (Muslims 0.7 per 100 py, non-Muslims 3.7 per 100 py) and in men circumcised at 13 years or older (Muslims 2.4 per 100 py and non-Muslims 4.7 per 100 py). However, these differences were not statistically significant (Mantel–Haenzel weighted RR = 0.29, CI 0.07–1.19). There were no significant differences in HIV incidence between the two religious groups after stratification for marital status, sexual behaviors, and the age of circumcision, although in all such strata the non-Muslims had higher HIV incidence rates. One noteworthy exception was a higher HIV incidence in Muslims who reported the use of alcohol (4.7 per 100 py), which suggests atypical behavior given Islamic proscriptions against alcohol consumption.

We also examined the distribution of HIV risk factors in circumcised Muslim and non-Muslim men. The Muslim men were significantly younger than circumcised non-Muslims (age 15–19 years: Muslims 24.3% (177/729) versus non-Muslims, 10.4% (18/173), $P < 0.0001$). Also, the Muslim men were circumcised at younger ages; 87.7% (639/729) of Muslims reported circumcision before puberty, compared with 48.0% (83/173) of non-Muslims ($P < 0.0001$). A higher proportion of Muslim men had never married (33.6%, 245/726), compared with non-Muslims (20.2%, 35/173, $P < 0.00001$). Muslims and non-Muslims reported similar frequencies of extramarital partners (33.5% and 37.8%, respectively, $P = 0.33$), and current condom use (10.8% and 10.4%, respectively, $P = 0.98$). However, Muslim men reported significantly less alcohol use (3.7%, 27/729), compared with non-Muslims (71.1%, 123/173, $P < 0.0001$).

In summary, as shown in Table 4, the incidence of HIV varied with these sociodemographic and behavioral characteristics, and the distribution of such characteristics differed by the religious affiliation of these circumcised men. We therefore used Poisson regression to estimate adjusted rate ratios of HIV acquisition associated with religion among circumcised men, adjusting for the risk factors in Table 4. The overall adjusted rate ratio of HIV acquisition among circumcised Muslim compared with circumcised non-Muslim men was 0.59 (CI 0.21–1.66). Among men circumcised at or before 12 years, the rate ratio of HIV acquisition was 0.69 (CI 0.15–3.16), and in men circumcised at 13 years or older, the rate ratio was 0.48 (CI 0.12–1.96). Among men circumcised before puberty, HIV incidence was 0.9/100 py in Muslims and 1.4/100 py in non-Muslims, compared with 1.8/100 py in uncircumcised men. Approximately half the protective effects of prepubertal circumcision may thus be attributable to circumcision *per se* (1.8–1.4 = 0.4/100 py), and approximately half to characteristics of Muslim men (1.4–0.9 = 0.5/100 py). Using the uncircumcised as the reference group, the adjusted rate ratio was 0.51 (CI 0.28–0.94) for Muslims, and 0.77 (CI 0.19–3.08) for non-Muslims. This analysis confined to circumcised men, suggests that Muslims may generally be at lower risk of HIV acquisition than non-Muslims, particularly in the age group 20–29 years. Although Muslims have a generally lower risk profile than circumcised non-Muslims, it is unclear what specific behaviors, other than abstinence from alcohol, might reduce the risk among Muslim men. However, key informant interviews suggest that the Islamic practice of post-coital cleansing before prayer may be an important factor explaining the lower incidence of HIV in circumcised Muslim men.

HIV acquisition in HIV-negative concordant couples and HIV acquisition and transmission in HIV-discordant couples

Linked spousal information was available for 2729 couples in which the male partner was initially HIV-negative. There were 530 HIV-negative circumcised

Table 4. HIV acquisition in HIV-negative circumcised Muslim and non-Muslim men.

	Circumcised Muslim HIV-negative men			Circumcised non-Muslim HIV-negative men			
	No.	Incident HIV cases/py	HIV incidence/ 100 py	No.	Incident HIV cases/py	HIV incidence/ 100 py	Rate ratio (95% CI)
All	729	13/1371	1.0	173	5/309	1.6	0.63 (0.2–2.7)
Age at circumcision (years)							
≤12	639	11/1197	0.9	83	2/144	1.4	0.64 (0.2–1.9)
> 12	90	2/174	1.2	90	3/165	1.8	0.63 (0.2–2.2)
Age (years)							
15–19	177	3/284	1.1	18	0/29	0	na
20–29	258	4/488	1.0	59	4/97	4.1*	0.24 (0.1–0.8)
30+	294	6/560	1.0	96	1/183	0.5	1.96 (0.4–8.7)
Marital status							
Never married	245	6/395	1.5	35	1/56	1.8	0.85 (0.2–3.7)
Monogamous	323	3/654	0.5	94	2/174	1.2	0.42 (0.1–1.4)
Polygamous	136	4/273	1.5	27	2/48	4.1	0.35 (0.1–1.1)
Divorced/separated/widowed	25	0/49	0	17	0/31	0	na
Sexual behaviors							
No extramarital partners	484	6/940	0.6	107	3/192	1.6	0.38 (0.2–1.1)
Extramarital partners	244	7/430	1.6	65	2/115	1.7	0.94 (0.3–2.8)
Current condom use	79	0/140	0	18	1/35	2.9	na
No condom use	650	13/1230	1.1	155	4/275	1.5	0.73 (0.3–1.6)
Alcohol past month	27	2/43	4.7	123	4/225	1.8	2.67 (0.8–8.3)
No alcohol	702	11/1328	0.8	50	1/84	1.2	0.67 (0.2–2.9)

CI, Confidence interval; py, person years.

men, and 49 of their wives were HIV positive (9.2%). Among 2199 HIV-negative uncircumcised men, 127 wives (5.8%) were HIV infected.

There were 2553 couples in which both partners were concordantly HIV negative. In 481 concordant HIV-negative couples the male partner was circumcised and nine seroconversions occurred over 967.8 py, giving an HIV incidence of 0.9/100 py. In 2072 concordant seronegative couples the male partner was uncircumcised, and there were 62 seroconversions over 4276 py, with an incidence of 1.5/100 py. This difference was not statistically significant (adjusted RR = 0.64, CI 0.39–1.05). There were 374 men with prepubertal circumcision married to HIV-negative wives, and seven seroconverted over 757.5 py, with an incidence of 0.9/100 py, which was not significantly lower than the rate in uncircumcised men (adjusted RR = 0.64, CI 0.37–1.10). Among concordant HIV-negative couples, circumcision did not significantly reduce the risk of male HIV acquisition, although the trend towards a protective effect was consistent with that observed in the general population.

There were 411 HIV-discordant couples. The proportion of circumcised men among couples in which the man was the HIV-positive partner was 13.0% (29/224), and this was lower than the proportion circumcised in discordant couples in which the man was the HIV-negative partner, 26.7% (50/187). This suggests that circumcision may be associated with a reduced risk of prevalent HIV infection in men (RR = 0.63, CI 0.46–0.85), consistent with our previously published findings of baseline results for the general population [10], and reports by other investigators [8,9]. This also suggests that the subgroup of HIV-discordant couples are not atypical with respect to circumcision status.

To address male acquisition risk we examined 187 couples in which the woman was the HIV-positive index partner (Table 5). Among these couples, there were no seroconversions in 50 HIV-negative circumcised men, whereas in 137 uncircumcised men, there were 40 seroconversions, with an HIV acquisition rate of 16.7 per 100 py (CI 11.9–21.4 per 100 py, $P = 0.0004$). Seventy-two per cent (36/50) of circumcised HIV-negative male partners were Muslims. Among the 14 non-Muslim circumcised men, no seroconversions were observed over 26.2 py, and this is significantly lower than the incidence of 16.7 per 100 py in uncircumcised non-Muslim men. The lower HIV incidence in circumcised men was statistically significant at all viral loads. In uncircumcised men, there was a significant trend of increased HIV incidence with a higher viral load in the female HIV-positive partner (χ^2 trend 11.5, $P = 0.0007$). This suggests a protective effect of circumcision on the risk of male HIV acquisition even under circumstances of high HIV exposure.

The 223 couples in which the man was the HIV-positive index partner were examined to assess HIV transmission (Table 5). The HIV transmission rate was 5.2 per 100 py if the man was circumcised, compared with 13.2 per 100 py if the man was uncircumcised. This difference was not statistically significant (unadjusted RR = 0.38, CI 0.13–1.22). However, for all HIV-positive male partners with viral loads of less than 50 000 copies/ml, no transmissions were observed in 22 circumcised men, compared with a transmission rate of 9.6 per 100 py (CI 6.1–13.1 per 100 py) in 143 uncircumcised men, and this difference was statistically significant ($P = 0.02$). At viral loads greater than 50 000 copies/ml, the transmission rates were similar in circumcised and uncircumcised HIV-infected men (25.0 and 25.6 per 100 py, respectively). The multivariate adjusted rate ratio of HIV transmission in circumcised versus uncircumcised HIV-positive men, adjusted for viral load was 0.41 (CI 0.10–1.14).

Table 5. HIV acquisition and transmission by circumcision status and viral load in discordant couples.

	Couples with circumcised men			Couples with uncircumcised men		
	No.	Incident HIV cases/py	HIV incidence/100 py (95% CI)	No.	Incident HIV cases/py	HIV incidence/100 py (95% CI)
			Male acquisition			Male acquisition
Male HIV− Female HIV−	50	0/106	0	137	40/239	16.7 (12.0–21.4)***
Viral load						
< 10 000	24	0/51	0	71	11/134	8.2 (3.6–12.9)***
10 000–49 999	18	0/37	0	46	20/72	27.8 (17.4–38.0)***
50 000+	8	0/18	0	20	9/33	27.3 (12.4–43.0)***
			Female acquisition			Female acquisition
Male HIV+ Female HIV−	29	3/58	5.2 (0–10.6)	195	46/349	13.2 (9.6–16.8)
Viral load						
< 10 000	12	0/24	0	73	10/144	6.9 (2.8–11.0)***
10 000–49 999	10	0/23	0	70	16/127	12.6 (6.8–18.4)***
50 000+	7	3/12	25.0 (0.5–49.5)	22	20/70	25.6 (15.4–35.8)

*** $P < 0.001$ based on confidence intervals of incidence rates.
CI, confidence interval; py, person years.

Muslims constituted 75% of circumcised HIV-positive male partners (21/28). In 21 circumcised, HIV-positive Muslim men, the transmission rate was 4.6 per 100 py (2/44 py) and in seven circumcised non-Muslims, there was one seroconversion over 11.9 py, with an HIV incidence of 8.4 per 100 py. The transmission rate was 13.2 per 100 py in 194 uncircumcised non-Muslims (46/348.7 py), but these rates by religion and circumcision status did not differ significantly from one another, partly as a result of small numbers.

Discussion

The findings from this representative community cohort suggest that circumcision may protect men from acquiring HIV infections (adjusted RR = 0.53, CI 0.33–0.87). The overall protective effects of circumcision observed in this study of a general population is comparable to some, but not all studies of other general populations in sub-Saharan Africa [8,9]. However, the general population protective effects are less than reported in prospective studies of self-selected high-risk populations (relative risks ranging from 0.12 to 0.4) [1–6,9]. The possibility that the magnitude of the protective effects of circumcision may be greater in self-selected subgroups at high risk of HIV is supported by our finding that circumcised HIV-negative men in discordant relationships with HIV-positive women experienced no seroconversions (Table 5) [7]. The absence of male HIV acquisition even if the HIV-positive female partner had a high viral load is striking, because we have previously shown that viral load is the main determinant of the risk of HIV infection among HIV-discordant couples [7]. A protective effect of circumcision on the risk of HIV acquisition is biologically plausible because the foreskin contains HIV target cells, the epithelium of the glans is thinner in uncircumcised men, and the prepuce may be more vulnerable to traumatic lesions during intercourse. Also, the preputial sac may be conducive to the survival of microorganisms [8,9,14]. In addition, circumcision may reduce the risk of genital ulcer disease and STD [1,7,8], which could act as co-factors for HIV infection [15].

Although our findings and those of other investigators suggest that circumcision may protect men from HIV acquisition, and that the magnitude of the effects may be comparable with other interventions such as STD control, we believe that the interpretation of these observational data on circumcision are complex. For example, reduced risks of HIV acquisition associated with circumcision were not found in subgroups such as non-Muslim men, adolescents and never-married men, or among men with STD symptoms or diagnoses (Table 2), and multivariate analyses of such subgroups, although constrained by small numbers, did not demonstrate any protection after adjustment (Table 3). Circumcision is not normative in these societies, and confounding by reason for circumcision is difficult to resolve. In the present study, religious affiliation is a major determinant of circumcision, and this presents a problem with analysis, because 80.8% of circumcised men were Muslim, and 99.1% of Muslim men were circumcised, whereas the prevalence of circumcision was only 3.7% in non-Muslims. The age of circumcision and reasons for circumcision also differed between these two religious groups. Among the 730 circumcised Muslims, 87.5% reported circumcision at or before 12 years of age, and all Muslims reported that circumcision was performed for religious reasons. In contrast, 49.7% of circumcised non-Muslims reported prepubertal circumcision, and 75% of non-Muslim men with post-pubertal circumcision indicated that the procedure was performed for health reasons. There are, therefore, fundamental differences between Muslims and non-Muslims in the prevalence, age of, and reasons for circumcision, which may confound associations with HIV risk in observational studies. We assessed the role of religion by comparing circumcised Muslim and non-Muslim men (Table 4). In general, circumcised Muslim men had lower HIV incidence than circumcised non-Muslims, and this was statistically significant for the age group 20–29 years. However, we could not identify specific characteristics or behaviors that might account for the lower HIV acquisition risk in Muslims. Therefore, we cannot exclude the possibility that the apparent protective effects of circumcision compared with the lack of circumcision in the general population, actually reflects subtle, unmeasured differences in risk behaviors between Muslim and non-Muslim men or their partners. For example, married Muslim men are predominantly polygamous, and polygamous unions may provide a closed sexual network reducing the risk of HIV introduction [16]. Also, Muslim men abstain from alcohol consumption, and alcohol is associated with high-risk behaviors. Key informant interviews suggest that penile hygiene may be important. Under Islam, individuals are considered unclean after intercourse, and Muslim men and women are required to perform post-coital ablutions. In addition, observant Muslims will often wash before daily prayer. Hygienic practices associated with religion may thus partly explain the protective effects of circumcision among Muslims. Similar difficulties of interpretation arise in other studies, such as in Kenya, where circumcision is only practised in selected ethnic groups that may have cultural practices that affect HIV risk [1]. Observational epidemiological methods may thus not be able to measure the relative contributions of highly correlated exposure characteristics [17], and it may be impossible to determine the effects on reduced HIV incidence caused by Islamic religion and culture, from the separate biological effects of circumcision *per se*.

Conversely, the effects of postpubertal circumcision may be underestimated, as a result of confounding by the indications for circumcision. In Rakai, 75% of postpubertal procedures were performed for health reasons, and men who have postpubertal circumcision for medical indications may have pre-existing pathology such as balanitis or phimoisis, secondary to STD or other genital infections. These previous infections are likely to be markers for high-risk behaviors that are in the causal pathway that places men at increased risk of HIV acquisition. Our estimates of the non-significant effects of postpubertal circumcision may thus be biased towards the null. Bias could also arise from the misclassification of reported circumcision status. We could not directly validate reported circumcision status by medical examination, but other African studies have shown a high level of agreement between reported and medically confirmed circumcision status [1,18].

The effects of circumcision on HIV risk in non-Muslims is also unclear. In the general population cohort, we observed no significant differences in HIV acquisition rates associated with circumcision status in non-Muslim men, irrespective of whether circumcision was performed before or after puberty (Table 2 and Table 4). However, among the couples with circumcised HIV-negative male partners in discordant relationships with HIV-infected women, there were 14 non-Muslim men who experienced no seroconversions, which suggests a protective effect independent or religion, albeit based on small numbers.

The overall effects of circumcision on HIV transmission from infected men to their HIV-negative partners was modest and not statistically significant (Table 5). However, it is noteworthy that there was no transmission if the circumcised HIV-positive men had viral loads of less than 50 000 copies/ml, whereas in uncircumcised HIV-positive men with viral loads of less than 50 000 copies/ml, the transmission rate was 9.6 per 100 py. Circumcision afforded no protection from HIV transmission at viral loads greater than 50 000 copies/ml (Table 5). Therefore, male circumcision may protect women from HIV transmission at lower, but not at higher, viral loads.

It has been suggested that circumcision might provide an appropriate intervention for HIV prevention [8,9], and some authors have advocated the promotion of widespread voluntary male circumcision [9]. However, at this juncture, we feel that such a policy may be premature. As noted above, observational epidemiological studies may not be adequate to measure the impact of circumcision on HIV risk. Our findings suggest that the protective effects of circumcision may be lower or negligible among certain subgroups such as non-Muslim men, adolescents and never-married men, and among men with postpubertal circumcision (Table 2). Moreover, the protective effects of circumcision on HIV acquisition appear to be less marked among men in the general population who have a lower intensity of HIV exposure, compared with the effects among highly exposed men in relationships with HIV-positive female partners (Table 2 and Table 5). Randomized clinical trials are needed to determine the utility of circumcision as an HIV preventative measure in a variety of settings. However, such trials present major difficulties in design and execution. For example, a trial of prepubertal procedures would entail the randomized circumcision of minors, which poses ethical issues, particularly with respect to parental consent and the provision of safe surgical procedures for large populations of young boys in these rural areas. In addition, if circumcision was performed before puberty, it would take many years of follow-up to observe an effect on male HIV acquisition, because male HIV incidence is relatively low until the mid-twenties in east African rural populations [19–21]. These considerations make the design of a clinical trial of prepubertal circumcision problematical. A trial of adult circumcision of men in discordant relationships may be more feasible, and the HIV incident endpoint could be determined within a reasonable time frame, but such a trial would present major ethical obstacles.

In summary, male circumcision may protect HIV-negative men from acquiring HIV infection to varying degrees. The effects were more modest in the general population, in which HIV exposure and incidence are relatively low. Also, the apparent protective effects of circumcision were not consistently observed in all subgroups and were largely associated with Muslim religious affiliation, which could be a marker for unmeasured differences in cultural practices or sexual behaviors. However, circumcision appears to be highly protective among HIV-negative men in a discordant relationship with an HIV-positive female partner, and circumcision may reduce HIV transmission from HIV-positive men with viral loads of less than 50 000 copies/ml. We believe that these observational data are not sufficient to justify the promotion of voluntary circumcision for HIV prevention in the general population or in high-risk groups, and that clinical trials are needed before policies on circumcision for HIV prevention can be established. In addition, studies of personal hygiene, particularly post-coital washing are warranted, because it may be simpler to clean the foreskin than to remove it.

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