THE B FILES

Case studies of bias in real life epidemiologic studies

Bias File 9. Male circumcision for HIV prevention: from confounding to causality

Compiled by

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Bias File 9. Male circumcision for HIV prevention

The story of male circumcision for HIV prevention is a long and fascinating one. Ecological studies in the 1980s and early 1990s provided early evidence that in African countries where male circumcision is practised, HIV seroprevalence was considerably lower than in areas where it is not practised. For example, Moses and others published an ecological study using data from 41 countries in Africa, with results supporting this hypothesis (figure). In the 1990s and early 2000s, a large number of observational studies were done, mostly cross-sectional studies (e.g. Auvert B et al. AIDS 2001), with a few case-control (e.g. Quigley M et al. AIDS 1997) and cohort (e.g. Gray RH et al. AIDS 2000) studies. Although many of these observational studies showed a protective effect of circumcision, they were highly controversial because of concerns about bias and confounding.

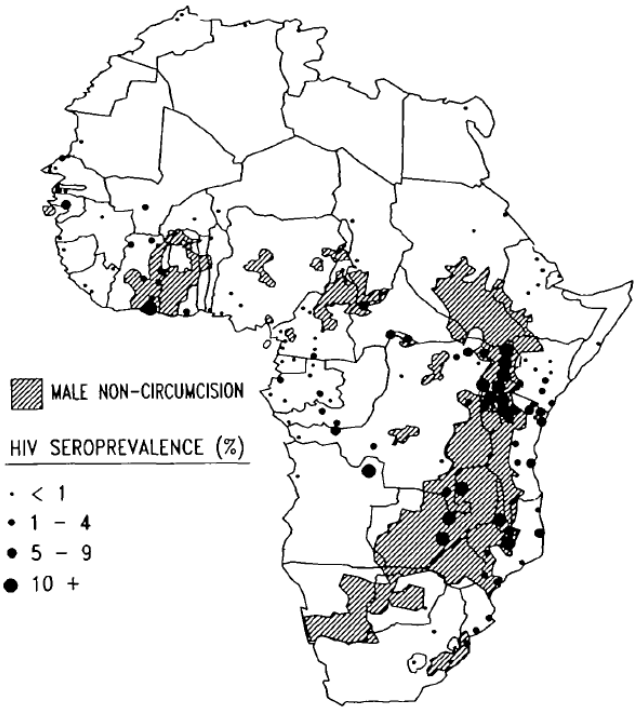


FIGURE 1. *Map of Africa showing political boundaries and usual male circumcision practice, with point estimates of general adult population HIV seroprevalence superimposed*

In 2005, a systematic review was published in the *Lancet Infectious Diseases* by Siegfried et al., and this review included 37 observational studies on circumcision and HIV. At that time, there were no published randomized controlled trials (RCTs) on this topic. The review concluded that "although most studies show an association between male circumcision and prevention of HIV, these results may be limited by confounding, which is unlikely to be adjusted for." The panel from the paper by Siegfried provides a list of potential confounding variables. Because of the risk of uncontrolled confounding, even in 2005, many researchers doubted whether the association between circumcision and HIV was really causal.

***Panel:* Potential confounding factors**

Age
Location of study (eg, rural, urban)
Religion
Education, occupation, and socioeconomic status
Sexual behaviour (eg, measured by age at first intercourse, number of sexual partners, contact with sex workers)
Any STIs
Condom use
Migration status
Travel to different countries
Other possible exposures (eg, injections, blood transfusions, homosexual intercourse)

In October 2005, the first ever randomized controlled trial on male circumcision and HIV was published in *PLoS Medicine* (Auvert B et al.). A total of 3,274 uncircumcised men in South Africa, aged 18–24 y, were randomized to a control or an intervention (circumcision) group with follow-up visits at months 3, 12, and 21. Male circumcision was offered to the intervention group immediately after randomization and to the control group at the end of the follow-up. The trial was stopped at the interim analysis. There were 20 HIV infections (incidence = 0.85 per 100 person-years) in the intervention group and 49 (2.1 per 100 person-years) in the control group, corresponding to an RR of 0.40 (95% CI: 0.24%–0.68%). This RR corresponds to a protection of 60% (95% CI: 32%–76%). The authors concluded that "male circumcision provides a degree of protection against acquiring HIV infection, equivalent to what a vaccine of high efficacy would have achieved."

This landmark first RCT on male circumcision provided powerful experimental evidence that the association between circumcision and HIV was unlikely to be due to confounding. The table below, from the *PLoS Med* paper, shows the distribution of various confounders in the two randomized groups. The groups were strikingly comparable, underscoring a critical advantage of randomization - known and even unknown and unmeasured confounders are likely to be evenly distributed across the intervention and control groups. Evidently, one well-conducted RCT had a bigger impact than a large number of observational studies.

Table 2. Baseline Characteristics of HIV-Negative Men Enrolled in the Trial

Background Characteristics		Control $n = 1,582$	Intervention $n = 1,546$
Age	Less than or equal to 21 y	52.4%	48.6%
	More than 21 y	47.6%	51.4%
Primary level of education completed		98.4%	98.3%
Religion	African traditional	47.0%	51.6%
	Protestant or Catholic	11.1%	11.9%
	Other religion	41.8%	36.5%
Ethnic group	Sotho	47.3%	49.0%
	Zulu	38.1%	32.8%
	Other	14.6%	18.2%
Drank alcohol in the past month		41.9%	42.2%
Reported sexual behaviour			
Have had first sexual experience		90.5%	91.8%
Median (IQR) age at first sex (years)[a]		16.6 (15.2–18.4)	16.8 (15.4–18.5)
Median (IQR) number of lifetime sex partners[b]		4 (2–7)	4 (3–7)
Used a condom at first sex[b]		13.4%	15.2%
Ever used a condom[b]		81.2%	82.3%
At-risk behaviour[c,d]		46.7%	46.8%
Married or living as married[d]		1.8%	1.8%
Mean (IQR) number of non-spousal partners[e]		1.4 (0–2)	1.4 (0–2)
At least one sexual partnership with only one sexual contact[e]		29.8%	30.7%
Mean (IQR) number of sexual contacts[e]		8.0 (0–8)	8.7 (1–8)
Attended a clinic for a health problem related to the genital area[e]		10.0%	9.6%

By 2007, two more RCTs on circumcision and HIV were fast-tracked and published in the same issue of the *Lancet*, one from Uganda (Gray et al. Lancet 2007) with 4996 men, and another from Kenya (Bailey et al. Lancet 2007) with 2784 men included. Both trials had to be stopped early because interim analyses showed significant efficacy. These trials confirmed the results of the first RCT from South Africa. In fact, the results of the 3 RCTs were stunningly consistent, as show in the meta-analysis by Mills et al. (HIV Med 2008).

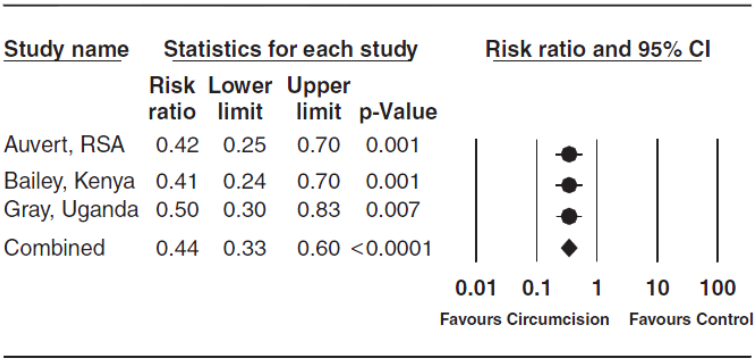
The table below (from Mills et al.) summarizes the results of all 3 RCTs:

Table 1 Study characteristics and outcomes

Study	Design	Setting	Population	*n*	Outcomes		Relative risk (95% confidence interval)
					Intervention	Control	
Auvert *et al.* (2005)*	Randomized trial	Orange farm, South Africa	Males aged 18–24 years	3128	20/1546	49/1582	0.42 (0.25–0.70)
Bailey *et al.* (2007)**	Randomized trial	Kisumu, Kenya	Males aged 18–24 years	2780	19/1388	46/1392	0.41 (0.24–0.70)
Gray *et al.* (2007)	Randomized trial	Rakai district, Uganda	Males aged 15–49 years	4996	22/2474	45/2522	0.50 (0.30–0.83)

*3274 randomized, 3128 included in analysis.
**2784 randomized, 2780 included in analysis.

The pooled random effects RR was 0.44 (95% CI 0.33–0.60). The risk difference was 0.014 (95% CI 0.07–0.21), yielding a NNT of 72 (95% CI 50–143) [Forest plot shown below, from Mills et al.].



Because of the strong and consistent evidence from RCTs, UNAIDS and other HIV agencies now recommend that male circumcision be recognized as an additional important intervention to reduce the risk of heterosexually acquired HIV infection in men. Using the RCT evidence, subsequent mathematical modeling studies by UNAIDS & WHO (*PLoS Med* 2009) suggest that one HIV infection can be averted for every five to 15 male circumcisions performed, and costs to avert one HIV infection ranges from US$150 to US$900 using a 10-y time horizon. The big challenge now is to actually scale-up and implement this efficacious intervention in countries where it is urgently needed. Unfortunately, even in 2009-2010, countries such as South Africa were not routinely providing this intervention, nor educating its public about the potential benefits of circumcision (Dugger C. *New York Times* 2009). Clearly, translating evidence and policy into real impact and saved lives is the next big step in this evolving story.

Another interesting twist in the tale is the lack of evidence that male circumcision offers the same degree of protection in homosexual men. In 2008, Millett et al. published a meta-analysis in *JAMA*, on whether male circumcision provides protection against HIV infection among men who have sex with men (MSM). In this meta-analysis of 15 observational studies of the association of circumcision status and HIV infection among 53 567 MSM, the odds of being HIV positive were 14% lower among MSM who were circumcised than among MSM who were uncircumcised, but the difference was not statistically significant. To date, no RCTs have looked the effect of circumcision among MSM.

For students of epidemiology, circumcision for HIV prevention offers a fascinating case study because this association has been studied using every possible epidemiological study design:

- Ecological (e.g. Moses et al., 1990)
- Cross-sectional (e.g. Auvert et al., 2001)
- Case-control (e.g. Quigley et al. 1997)
- Cohort (e.g. Gray et al. 2000)
- Systematic review of observational studies [before RCTs were done] (Seigfried et al. 2005)
- Randomized controlled trials (Auvert 2005; Gray 2007 & Bailey 2007)
- Meta-analysis of RCTs (Mills et al. 2008).

A complete set of these various designs is included in the appendix. Together, these papers provide real-life examples of every major epidemiologic concept, from study design, measures of disease frequency and effect, to selection bias, information bias, confounding, interaction, meta-analysis and causality. Also, this collection provides an interesting example of RCT evidence agreeing with much of the observational evidence. Not only was confounding not the reason for the association seen in the observational studies, but in fact there didn't turn out to be very much confounding at all. The RCT effects turned out to be fairly similar in magnitude to most of the case-control and cohort estimates, suggesting that there was almost no confounding at all. Finally, this collection provides a nice example of epidemiology succeeding in uncovering a real causal effect, one that can be a useful public health intervention for an important disease for which an effective vaccine is still not available.

Sources and suggested readings*

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*From this readings list, the most relevant papers are enclosed.

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Geographical Patterns of Male Circumcision Practices in Africa: Association with HIV Seroprevalence

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Moses S (Department of Medical Microbiology, University of Nairobi, PO Box 19676, Nairobi, Kenya), Bradley J E, Nagelkerke NJD, Ronald A R, Ndinya-Achola J O and Plummer F A: Geographical patterns of male circumcision practices in Africa : Association with HIV seroprevalence. *International Journal of Epidemiology* 1990; **19**: 693–697.
To ascertain whether male circumcision might explain some of the geographical variation in human immunodeficiency virus (HIV) seroprevalence in Africa, we investigated the association between the practice of male circumcision at a societal level and HIV seroprevalence. Male circumcision practices for over 700 African societies were identified, and HIV seroprevalence in general adult populations from 140 distinct locations in 41 countries was obtained. In locations where male circumcision is practised, HIV seroprevalence was considerably lower than in areas where it is not practised. This study supports the hypothesis that lack of circumcision in males is a risk factor for HIV transmission.

The seroprevalence of human immunodeficiency virus (HIV) in the general population in Africa varies considerably both between and within countries, ranging from being undetectable in some areas to over 20% in others.[1–4]. This variation may reflect differences in sexual exposure, including numbers of sexual partners and the frequency of sexual intercourse. Another explanation may be that due to its relatively recent introduction into Africa, HIV is still in the process of diffusion and has not yet reached an equilibrium state.

Factors have been postulated which may be responsible for facilitating the spread of HIV infection by increasing the infectivity of carriers of the virus or the susceptibility of individuals to acquiring it.[5–8]. Lack of circumcision in males is one of the factors that has been implicated in increasing susceptibility to HIV infection. In this study we have demonstrated a geographical association between HIV seroprevalence and male circumcision practices at a societal level in Africa, lending further support to this hypothesis.

METHODS

Several ethnographic data sources were reviewed, and male circumcision practices for over 700 African societies (ethnic groups) were ascertained.[9–15] The database of the Human Relations Area Files in Yale, Connecticut, was a major source of information. All societies whose circumcision status we could identify were mapped using Murdoch's classification of African societies.[16] Some of the information is old and there may be situations where circumcision practices have changed. However, these practices tend to be deeply ingrained in African societies, and it is unlikely that overall they will have changed significantly. We have assumed that within a given area, circumcision practice either way is essentially universal. This may not always be the case, particularly in urban areas. However, there are only a few countries in Africa in which there is a significant mixture of societies which practise and do not practise male circumcision, so our assumption seems reasonable.

Seroprevalence data were collected from several sources. The US Bureau of the Census provided access to their data base on HIV seroprevalence, which contains information drawn from all available sources. We also scanned published scientific literature, abstracts from scientific conferences and government reports.[17–22] We limited our analysis to studies conducted in 1986 or afterwards with a sample size of at least 100. We excluded studies involving prostitutes, barmaids or barmen, long distance truck drivers, hos-

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pitalized patients, patients with diseases associated with HIV infection and prisoners. We included studies involving general community members, students over 15 years of age, general worker populations, antenatal clinic attenders and blood donors. Data on HIV–1 and HIV–2 were combined. Where studies in the same area at the same time showed differing results, an average was used. In areas where studies were conducted in the same population at different times, we took the most recent result. Therefore, only one seroprevalence figure was associated with each geographically distinct location.

The resulting dataset contains male circumcision and HIV seroprevalence information from 140 geographically distinct locations in 41 different countries. Seventy-two (51%) of these locations were considered to be urban and 68 (49%) rural. To establish urban/rural status we used the description of the location given in the original study, or made an inference from the information provided. Study areas described in general terms, such as district or province, were considered to be rural.

RESULTS

The map of male circumcision practices in Africa is shown in Figure 1. The blank areas are those in which male circumcision is generally practised, and the hatched areas where male circumcision is not practised. There are a few societies for which we could not determine a male circumcision status, and for the sake of clarity we left the corresponding areas blank as well. However, no locations from which we had seroprevalence data were situated in those areas, so the analysis is not affected.

There is a large belt in eastern/central Africa in which male circumcision is not practised, beginning in southern Sudan and extending south, covering most of Uganda, parts of western Kenya and western Tanzania, virtually all of Rwanda, Burundi, Zambia, Malawi and Zimbabwe, and parts of Botswana, Namibia, Mozambique and South Africa. There is another large area of 'non-circumcision' covering central/eastern Ivory Coast and western/central Ghana. This is the only large area of non-circumcision in West Africa. There appears to be a non-circumcised population in northern Cameroon/western Central African Republic (CAR), and a small area where male circumcision is not practised along the Ubangi River in southern CAR/northern Zaire.

The locations of the HIV seroprevalence data points were superimposed on the circumcision map (Figure 1). Each data point is represented by a circle, the largest circle representing HIV seroprevalence rates of over 10% and the smallest circle rates of less than 1%. It can be seen that the larger circles cluster in the non-circumcised areas and the smaller circles in the circumcised areas. Variations in HIV seroprevalence and in male circumcision status both within and between countries can be examined. A number of interesting points emerge:

There is a tendency for urban areas to be associated with higher levels of HIV seroprevalence than rural areas. This depends to some extent, however, on whether the urban or rural area is within a circumcised or non-circumcised zone. In the large non-circumcised belt of eastern/central Africa, HIV seroprevalence is high in both urban and many rural areas. Of the data points from circumcised and non-circumcised areas 53% (51/96) and 48% (21/44) were urban respectively. This difference was not statistically significant (Chi-square = 0.34, $P > 0.5$).

In Tanzania and Kenya, the areas of high HIV seroprevalence in the west are also areas where circumcision is not practised, in contrast to other areas of the two countries where seroprevalence is lower, and where male circumcision is the norm.

The major pocket of non-circumcision in West Africa, corresponding to parts of Ivory Coast and Ghana, is associated with higher levels of HIV seroprevalence than elsewhere in West Africa, with the exception of Guinea-Bissau. Circumcision is almost universally practised elsewhere in West Africa, including Nigeria, and there HIV seroprevalence is almost uniformly low.

Most societies in South Africa and the more populous areas of Botswana practice circumcision. Studies among mine workers in South Africa have shown low rates of HIV seroprevalence among South Africans and Botswanians, but higher rates among migrant workers, particularly Malawians, among whom male circumcision is generally not practised.[23]

Most societies in Zaire practice male circumcision. Seroprevalence in the general population in Kinshasa, the capital city, is relatively high, but not as high as in other cities (and even some rural areas for that matter) in the eastern/central Africa non-circumcised belt. Other areas in Zaire with higher seropositivity rates (northeastern Zaire, Kivu and Lubumbashi) border on Uganda, Rwanda and Zambia, and are in fact areas in which male circumcision is generally not practised. HIV seroprevalence in rural areas in Zaire from which we have data is generally low.

Finally, it should be noted that Guinea-Bissau, the Republic of the Congo and parts of Angola are somewhat anomalous in this context, in that male circumcision is generally practised, but these countries exhibit moderately high levels of HIV seroprevalence.

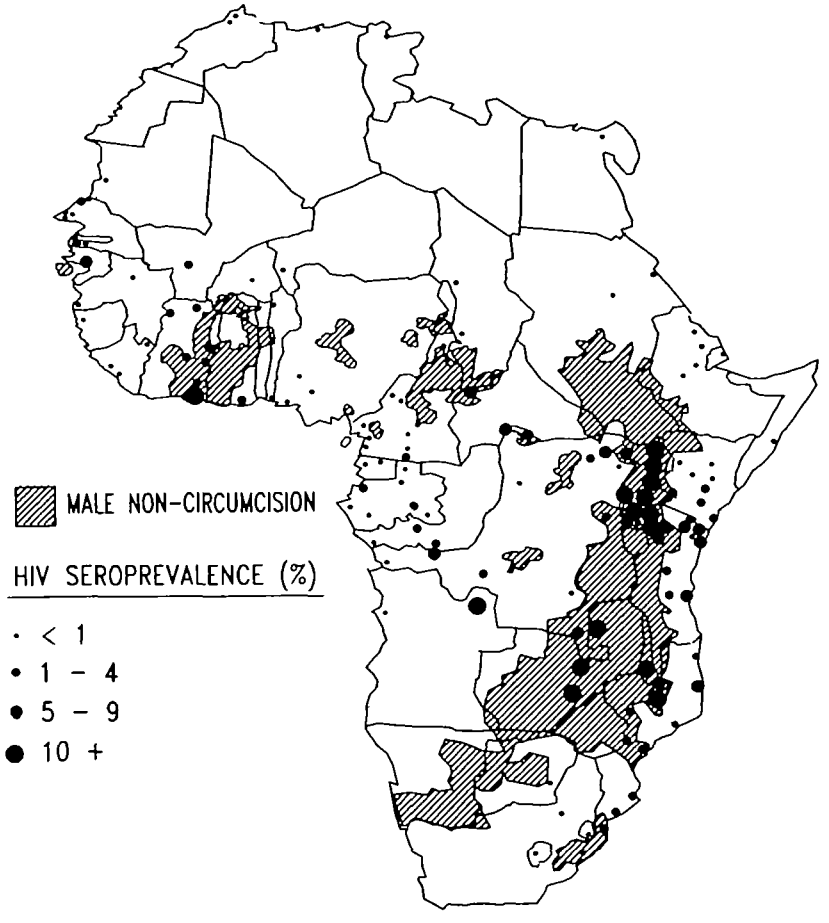

FIGURE 1. *Map of Africa showing political boundaries and usual male circumcision practice, with point estimates of general adult population HIV seroprevalence superimposed*

The group of data points from non-circumcised areas had a mean HIV seropositivity of 7.37%, compared with 1.41% in points from circumcised areas, giving a ratio of 5.2:1. Table 1 compares male circumcision status with HIV seroprevalence divided into four categories: less than 1%, 1–4%, 5–9%, and over 10%. Of the 68 data points associated with a seroprevalence of less than 1% (taken from 31 different countries), all but two occur in areas where circumcision is practised. Conversely, of the 17 data points with a seroprevalence of over 10% (which are taken from seven different countries), all but one occur in areas where circumcision is not practised.

The seroprevalence observations are clearly not independent, and there is a high degree of within-country ('intra-class') correlation. To eliminate this effect, we calculated the ratio between mean HIV seroprevalence in data points from 'circumcised' and 'non-circumcised' areas, in countries with a mixture of both. There are five countries from which we have such data: Côte D'Ivoire, Kenya, Mozambique, Tanzania and Zaire. There is a tendency for this ratio to be greater than unity (Table 2). To test this tendency statistically, we ranked all seroprevalence observations for each of the five countries separately, and calculated the differences between the expected and observed

TABLE 1 *General adult population HIV seroprevalence and male circumcision status in 140 geographically distinct locations in Africa*

Male circumcision status	General adult population HIV seroprevalence (%)				
	<1	1-4	5-9	10+	Total
Practised	66	21	8	1	96
Not practised	2	18	8	16	44
Total	68	39	16	17	140

TABLE 2 *Countries with both uncircumcised and circumcised areas: Ratio of mean general adult population seroprevalence values*

Côte D'Ivoire	2.4
Kenya	4.5
Mozambique	0.9
Tanzania	2 3
Zaire	2.0

rank sums, summed over the five countries, divided by the standard deviation. The value of the resulting approximately normally distributed test statistic was 2.75, $P<0.01$.

DISCUSSION

In this ecological study, large differences in HIV seroprevalence were found between populations practising male circumcision and populations where circumcision is not practised. These differences must be attributable either to circumcision, or to some confounding factor or factors.

There is considerable evidence in the literature supporting an association between the presence of a foreskin and susceptibility to sexually transmitted diseases (STDs), particularly to those causing genital ulceration.[24-26] Clinical studies in Kenya and the US have also found a relationship between lack of circumcision in men, and both HIV seroprevalence and incidence.[27,28] Viral entry may be facilitated by microtraumatic lesions of the foreskin sustained during normal sexual intercourse or by mini-ulcerations of the foreskin caused by recurrent balanitis.[29,30] In one study of male STD clinic attenders in New York City, no association between circumcision status and HIV infection was observed.[31] However, although homosexuality and intravenous drug abuse were controlled for in the analysis, it is likely that only a small proportion of infections were acquired through heterosexual intercourse, the mode of transmission of interest in this context, resulting perhaps in insufficient power to detect such an association.

In a recent study, Bongaarts *et al.* found a high correlation between estimates of adult HIV seroprevalence in capital cities of African countries and the proportion of circumcised males at a national level, supporting our findings.[32] The problem in the Bongaarts study of correlating variables with different units of analysis (national averages for circumcision data and capital city estimates for HIV seroprevalence) has been avoided in our study. Furthermore, mapping discrete HIV seroprevalence points has allowed us to examine variations in circumcision status and HIV seroprevalence both within and between countries and regions.

Possible confounders for the association observed in this study must be considered. HIV seroprevalence tends to be higher in urban than in rural areas, but we have shown that the urban/rural location of the data points in this study are evenly distributed across circumcised and non-circumcised areas. HIV seroprevalence also increases with time, but only data from recent seroprevalence studies were considered. Another possible confounder is the place of introduction of HIV. If the virus were first introduced into an uncircumcised area and then later diffused into circumcised areas, considerable differences in HIV seroprevalence could be caused by time lag alone. However, the association between HIV infection and circumcision is seen within individual countries in which both practices occur. It is possible that HIV may have been introduced first within a given country into an area where male circumcision is not practised, but it is unlikely that this would be so for several different countries. There is no evidence of which we are aware that patterns of sexual behaviour can either be excluded or included as a confounder, but this and other unknown confounders that cannot be addressed in an ecological study could influence the observed association. More studies are required, therefore, which investigate the association between HIV infection and male circumcision status at the level of the individual. Such studies should ideally be undertaken in countries such as Tanzania and Mozambique, where HIV seroprevalence is relatively high, and male circumcision practices vary.

Circumcision practices tend to be deeply rooted within African societies, and will not easily be amenable to change. However, if the lack of male circumcision is indeed an important risk factor for HIV infection, then it merits some consideration as a possible intervention in the control of HIV transmission.

ACKNOWLEDGEMENTS

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Male circumcision and HIV infection in four cities in sub-Saharan Africa

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Objectives: To explore the role of male circumcision in the spread of HIV infection in four urban populations in sub-Saharan Africa.

Design and methods: A cross-sectional population based study was conducted in four cities in sub-Saharan Africa with different levels of HIV infection. HIV prevalence among adults was relatively low in Cotonou (Benin) and in Yaoundé (Cameroon), and exceeded 25% in Kisumu (Kenya) and in Ndola (Zambia). In each city, a random sample was taken of men and women aged 15–49 years from the general population. Consenting study participants were interviewed about their socio-demographic characteristics and their sexual behaviour, and were tested for HIV, herpes simplex virus type 2, syphilis, gonorrhoea and chlamydial infection. Men underwent a genital examination.

Results: In Cotonou and in Yaoundé, the two low HIV prevalence cities, 99% of men were circumcised. In Kisumu 27.5% of men were circumcised, and in Ndola this proportion was 9%. In Kisumu, the prevalence of HIV infection was 9.9% among circumcised men and 26.6% among uncircumcised men. After controlling for socio-demographic characteristics, sexual behaviour and other sexually transmitted infections, the protective effect of male circumcision remained with an adjusted odds ratio of 0.26 (95% confidence interval = 0.12–0.56). In Ndola, the prevalence of HIV infection was 25.0% in circumcised men and 26.0% in uncircumcised men. The power was insufficient to adjust for any differences in sexual behaviour.

Conclusions: The differences in epidemic spread of HIV are likely to be due to differences in the probability of transmission of HIV during sexual exposure as well as differences in sexual behaviour. Male circumcision is one of the factors influencing the transmission of HIV during sexual intercourse, and this study confirms the population level association between HIV and lack of male circumcision, as well as a strong individual level association in Kisumu, the only city with sufficient power to analyze this association.

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Keywords: HIV, Africa, male circumcision

Introduction

The role of male circumcision in the spread of HIV in sub-Saharan Africa was first suggested more than 10 years ago [1]. Ecological studies found a correlation between the practice of male circumcision and the prevalence of HIV infection in the general population that suggested that HIV was more prevalent in areas in sub-Saharan Africa where male circumcision was not practised than in areas where men were circumcised

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[2,3]. There were several limitations to these studies. The high HIV prevalence in some areas may have been due to an earlier start of the epidemic, and no allowance was made for possible differences in sexual behaviour and in other sexually transmitted infections (STIs) between the different population groups [4].

In addition, several epidemiological studies were conducted in Africa that included male circumcision as a potential risk factor for HIV infection at the individual level. These studies allowed for differences in sexual behaviour but came up with varying strengths of the association between circumcision and HIV risk. A recent systematic review and meta-analysis of these studies showed a highly significant protective effect overall with the strongest effect in populations at high risk for HIV and STIs [5].

In the multicentre study on factors determining the differential spread of HIV in African cities, we took male circumcision into consideration as one of the possible factors that could explain the differences in rate of spread of HIV between Cotonou (Benin), Yaoundé (Cameroon), Kisumu (Kenya) and Ndola (Zambia). This paper presents the results of the analyses of the association between male circumcision and HIV.

Methods

The methods of the study are described in detail elsewhere [6]. Briefly, the study took place in four cities in sub-Saharan Africa: two with a relatively low and stable prevalence of HIV (Cotonou, Benin and Yaoundé, Cameroon), and two with a high prevalence of HIV (Kisumu, Kenya and Ndola, Zambia). We believe that the differences in prevalence between these four cities were due to differences in rate of spread of HIV rather than differences in time since the start of the epidemics. In each of the four cities, a random sample was taken of about 2000 adults aged 15–49 years. Consenting men and women were first interviewed about their socio-economic background and sexual behaviour. The questionnaire on sexual behaviour also included a section on characteristics of any non-spousal partners in the past 12 months. Men were interviewed about their circumcision status, age at circumcision, symptoms suggestive of a STI in the past 12 months, and health-seeking behaviour for STIs.

After the interview, men and women were asked to provide a blood sample, which was tested for HIV, herpes simplex virus type 2 (HSV-2) and syphilis, and a urine sample, which was tested for gonorrhoea and chlamydial infection by DNA amplification techniques. Men underwent a genital examination to confirm the reported circumcision status and to check for any signs of STI (mainly genital ulceration and urethral discharge). The circumcision status was recorded as 'circumcised', 'not circumcised' or 'uncertain circumcision status'. Nineteen men in Cotonou and one man in Ndola and in Kisumu had uncertain circumcision status and were excluded from the analyses.

All data were double-entered and validated in EPI-INFO version 6.04a (CDC, Atlanta, Georgia, USA). Further data cleaning, and data analysis was carried out with SPSS version 8.0 for Windows (SPSS, Inc, Chicago, Illinois, USA). The analyses presented in this paper are restricted to men who reported that they had ever had sex. The proportions of men who were circumcised and the age at circumcision were compared across the four cities. For each city, the association between circumcision and HIV infection was explored. Where the power was sufficient, multivariate regression analyses were conducted. The following variables were considered as potential confounding factors: socio-demographic characteristics (age, educational attainment, occupation, religion, ethnic group, travel in the past 12 months), sexual behaviour (age at first sexual intercourse, marital status, lifetime number of sex partners, number of non-spousal partners in the past 12 months, one-off sexual contacts or contacts with a sex worker in the past 12 months), and other STIs (HSV-2 infection, syphilis, gonorrhoea and chlamydial infection). Variables were selected for inclusion in the logistic regression model using the forward stepwise procedure. Variables that were associated with HIV infection at a significance level of 0.15 or less were entered into the model. In the final model, only those variables were retained that were associated with HIV infection at a significance level of 0.05 or less.

To exclude the possibility that the lower risk of HIV infection in circumcised men is due to the fact that their partners are less HIV infected than the partners of uncircumcised men, estimates were made of the HIV prevalence in spousal and in non-spousal partners of both groups of men. The reported characteristics of non-spousal partners (ethnic group, age and marital status) were compared between circumcised men and uncircumcised men. Using the distribution of these characteristics and the data on HIV infection in women who reported non-spousal partnerships in the past 12 months, the prevalence of HIV infection was estimated in the non-spousal partners of men. As for the spouses, the comparison was made between spouses of HIV-negative circumcised men and HIV-negative uncircumcised men.

Results

Prevalence of circumcision in the four cities and its association with HIV infection

Table 1 presents the numbers of men who were interviewed and the numbers who were examined, in each city. In Cotonou and Yaoundé, there was excellent agreement between interview reports of being circumcised and clinical examination, but almost all men who

Table 1. Circumcision status as reported by men and as ascertained by clinical examination

	Cotonou	Yaoundé	Kisumu	Ndola
Total number of men interviewed	1021	973	829	720
Number of men who had had sex	894	882	765	642
Number of sexually active men who were examined	767	784	568	512
% of men who reported being circumcised and were confirmed circumcised on clinical examination	723/729 (99.2%)	755/761 (99.2%)	148/164 (90.2%)	41/56 (73.2%)
% of men who reported not being circumcised and were confirmed not circumcised on clinical examination	1/18 (5.6%)	1/22 (4.5%)	394/402 (98.0%)	448/453 (98.9%)

Table 2. Circumcision status as ascertained by clinical examination and prevalence of HIV infection by circumcision status

	Cotonou	Yaoundé	Kisumu	Ndola
% circumcised	741/748 (99.1%)	777/784 (99.1%)	156/567 (27.5%)	46/511 (9.0%)
% HIV-positive[a]				
Circumcised	27/735 (3.7%)	35/775 (4.5%)	14/141 (9.9%)	11/44 (25.0%)
Uncircumcised	0/7 (0%)	1/7 (14.3%)	96/361 (26.6%)	117/450 (26.0%)
Odds ratio (95% confidence interval)	51 (0 to > 100)	0.3 (0.03–2.4)	0.3 (0.2–0.6)	1.0 (0.5–1.9)
Power (%) to detect a twofold difference in HIV prevalence between circumcised men and uncircumcised men	9	11	91	48

[a] The total figures of men who were and were not circumcised do not tally with the figures in the top row because not all men were tested for HIV.

said they were not circumcised were in actual fact circumcised. In Kisumu and Ndola, there was very good agreement between reports of not being circumcised and clinical examination (> 95% agreement). Men who said they were circumcised, however, misreported their circumcision status in 9.8 and 26.8% of cases, respectively. In the further analyses, the differentiation between circumcised men and uncircumcised men was made based on the clinical examination results.

In Cotonou and Yaoundé, the two low HIV prevalence cities, almost all men were circumcised (Table 2). In these cities, almost all men (> 99%) reported being circumcised before becoming sexually active. In Ndola, only 9.0% of men were circumcised, of whom 87% (34/39) were circumcised before their first sexual experience. In Kisumu, 27.5% of men were circumcised, of whom 83% (120/145) were circumcised before they became sexually active. The median age at circumcision was 4 years in Cotonou and Yaoundé, 11 years in Kisumu and 10 years in Ndola. The proportion of men circumcised after the age of 20 years was 0.0% in Cotonou and Ndola, 0.1% in Yaoundé, and 8.3% in Kisumu.

In each of the four cities, circumcision is practised primarily out of cultural preference rather than because of religious affiliation. In Cotonou and Yaoundé, circumcision is practised by all ethnic groups. In Kisumu only 10% of Luo men, the predominant ethnic group, were circumcised compared with 85% of men belonging to other ethnic groups (mainly Luya). In Ndola, 4–5% of men belonging to the two main ethnic groups, Bemba and Nyanja, were circumcised compared with 24% of men belonging to the other ethnic groups. Of the circumcised men in Cotonou, Yaoundé and Kisumu, approximately 13% were Muslim; in Ndola, this proportion was 7%.

Table 2 presents the HIV prevalence in men by circumcision status. In Kisumu, the prevalence of HIV infection was significantly lower in circumcised men (9.9%) than in men who were not circumcised (26.6%). In Cotonou and Yaoundé, there were seven uncircumcised men, of whom none and one, respectively, were HIV seropositive. In Ndola, the prevalence was about the same in the two groups. Figure 1 presents the HIV prevalence in the different ethnic groups in the four cities by the proportion of men who are circumcised. The HIV prevalence is negatively correlated with the proportion of men who are circumcised (Spearman correlation = –0.85, $P = 0.000$, $n = 14$).

In Kisumu, the prevalence of HIV infection was 5.5% in men who were circumcised before first sexual intercourse, 26.1% in men who were circumcised after age at first sex, and 26.6% in uncircumcised men. The difference in prevalence between men who were circumcised before and after first sexual intercourse was statistically

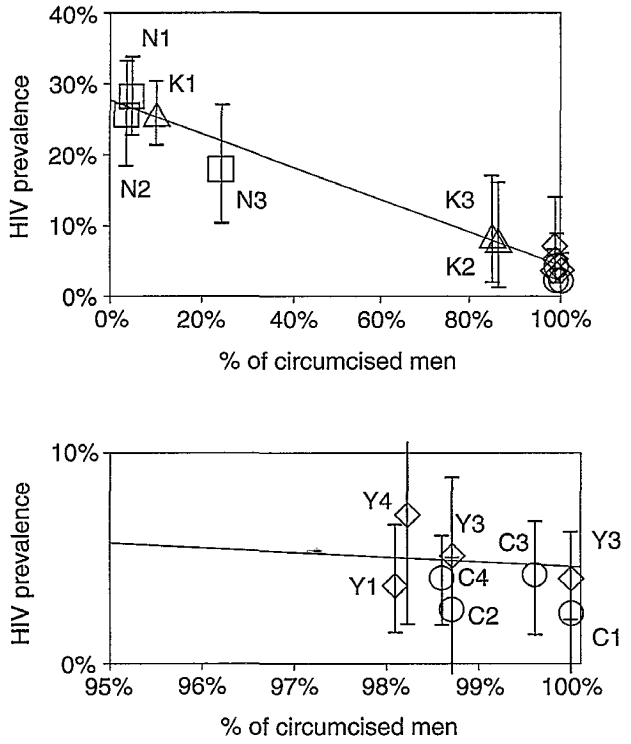

Fig. 1. HIV prevalence versus proportion of circumcised men in each ethnic group of men (see text) in Cotonou (A), Yaoundé (H), Kisumu (B) and Ndola (G). In each figure, the regression line has been drawn. The ethnic groups are: in Ndola: N1, Bembas; N2, Nyanjas; N3, others; in Kisumu: K1, Luos; K2, Luyas; K3, others; in Yaoundé: Y1, Bassas & bakokos; Y2, Pahouins; Y3, Bamilekes; Y4, others; in Cotonou: C1, Ninas; C2, Gouns; C3, Fons; C4, others.

significant ($P = 0.007$). In Ndola, one of the five men who were circumcised after age at first sex was HIV infected, compared with 20.5% (8/39) of the men who were circumcised before first sexual intercourse. In the further analyses, the circumcised men include all men who were circumcised regardless of the age at which it was performed.

Kisumu was the only city where the power was sufficient to allow multivariate analysis of circumcision as a risk factor for HIV infection (Table 2).

Male circumcision as a protective risk factor for HIV infection in Kisumu

In Kisumu, circumcised men were less likely to have had their first sexual experience before age 15 than men who were not circumcised; they had a higher educational attainment and were more likely to have a full-time job (Table 3). Luo men were under-represented among circumcised men, as circumcision is traditionally not practised by the Luo. Almost all Muslims were circumcised. There were no statistically significant differences between circumcised men and uncircumcised men in marital status, lifetime number of sex partners, number of non-spousal partners in the past 12 months, one-off sexual contacts and contacts with sex workers in the past 12 months, alcohol consumption and condom use. However, circumcised men had less HSV-2 infection and syphilis, and were less likely to report an episode of STI in the past 12 months than men who were not circumcised.

Table 3 also presents the odds ratios (OR) for HIV infection associated with circumcision status, stratified on each of the variables used in the comparison between circumcised men and uncircumcised men. In these stratified analyses, there was no evidence for confounding of the protective effect of male circumcision by any of these variables. There was also no significant interaction.

Table 4 presents the results of the univariate analysis of risk factors for HIV infection, in all men and in Luo men only. Apart from circumcision status, the following socio-demographic and behavioural variables were significantly associated with risk of HIV infection: age, marital status, lifetime number of sex partners, number of non-spousal partners in the past 12 months, alcohol consumption and occupation. Men with HSV-2 infection or syphilis and men who reported STI symptoms in the past 12 months were significantly more likely to be HIV infected. In the logistic regression model including the socio-demographic and behavioural variables circumcision status, marital status and alcohol consumption were independent risk factors for HIV infection (Table 5). After adding the STI variables to the model, marital status was no longer significantly associated with HIV risk. Circumcision status, alcohol consumption, HSV-2 infection and history of STI symptoms in the past 12 months remained independent risk factors for HIV infection. The results were similar for all men and for Luo men only: in the final model including the STI variables, the OR was 0.26 [95% confidence interval (CI) = 0.12–0.56] and 0.21 (95% CI = 0.06–0.78), respectively.

Information was available on 329 non-spousal partners of uncircumcised men and 133 non-spousal partners of men who were circumcised. Table 6 compares a few characteristics of these partners, who were used to estimate the prevalence of HIV among the partners of both groups of men. Circumcised men tended to have older partners and were less likely to have Luo partners than uncircumcised men. The estimated prevalence of HIV infection among the partners of both groups of men was the same (32%).

Data were available on 117 spouses of HIV-negative men in Kisumu. Of the spouses of circumcised men, 9.7% (3/31) were HIV infected, whereas 18.6% (16/86) of spouses of uncircumcised men were HIV infected. The difference was not statistically significant ($P = 0.2$).

Table 3. Comparison between men in Kisumu who are and men who are not circumcised

	Not circumcised, *n* (%)	Circumcised, *n* (%)	Odds ratio (95% confidence interval) for association between HIV and circumcision
Sample	361 (100)	141 (100)	0.30 (0.18–0.55)
Age			
15–19 years	77 (21.3)	20 (14.2)	0.96 (0.10–9.1)
20–29 years	131 (36.3)	66 (46.8)	0.31 (0.12–0.78)
30–39 years	97 (26.9)	39 (27.7)	0.15 (0.05–0.45)
40–49 years	56 (15.5)	16 (11.3)	0.48 (0.12–1.9)
	$P = 0.08$		
Marital status			
Never married	151 (41.8)	59 (42.1)	0.16 (0.02–1.2)
Ever married	210 (58.2)	81 (57.9)	0.30 (0.16–0.59)
	$P = 1.0$		
Age at first sex			
> 14 years	243 (67.3)	108 (77.7)	0.27 (0.13–0.56)
≤ 14 years	118 (32.7)	31 (22.3)	0.31 (0.09–1.1)
	$P = 0.03$		
Number of lifetime partners			
1–3	101 (28.0)	42 (29.8)	0.31 (0.07–1.4)
4–9	154 (42.7)	47 (33.3)	0.38 (0.15–0.95)
> 9	47 (29.4)	52 (36.9)	0.22 (0.09–0.57)
	$P = 0.13$		
Number of non-spousal partners in past 12 months			
0	175 (48.5)	60 (42.6)	0.23 (0.10–0.53)
1	108 (29.9)	49 (34.8)	0.47 (0.17–1.3)
> 1	78 (21.6)	32 (22.7)	0.41 (0.08–1.9)
	$P = 0.46$		
One-off contact or commercial sex in past 12 months			
0	338 (93.6)	131 (92.9)	0.32 (0.17-0.58)
≥ 1	23 (6.4)	10 (7.1)	–
	$P = 0.84$		
Alcohol			
< Once a month	239 (66.2)	122 (62.4)	0.36 (0.16–0.84)
≥ Once a month	122 (33.8)	53 (37.6)	0.21 (0.09–0.52)
	$P = 0.47$		
Education			
No primary	96 (26.6)	29 (20.6)	0.21 (0.05–0.95)
Primary complete	154 (42.7)	47 (33.3)	0.48 (0.20–1.2)
Secondary/higher	111 (30.7)	65 (46.1)	0.23 (0.08–0.61)
	$P = 0.005$		

Continued overleaf

Table 3. Continued

	Not circumcised, *n* (%)	Circumcised, *n* (%)	Odds ratio (95% confidence interval) for association between HIV and circumcision
Occupation[a]			
Full time	77 (21.3)	62 (44.0)	0.31 (0.13–0.74)
Student	58 (16.1)	8 (5.7)	
Other	226 (62.6)	71 (50.4)	0.21 (0.09–0.51)
	$P < 0.001$		
Ethnic group			
Luo	345 (95.6)	45 (31.9)	0.33 (0.13–0.86)
Other	16 (4.4)	96 (61.9)	1.6 (0.18–13.1)
	$P < 0.001$		
Religion			
Christian	309 (85.6)	107 (75.9)	0.33 (0.17–0.64)
Muslim	1 (0.3)	18 (12.8)	
Other	51 (14.1)	16 (11.3)	0.15 (0.018–1.2)
	$P < 0.001$		
Travel in the past 12 months			
< 2 trips	137 (41.9)	43 (33.3)	0.41 (0.16–1.0)
> 1 trip	190 (58.1)	86 (66.7)	0.23 (0.10–0.54)
	$P = 0.11$		
Herpes simplex virus type 2			
Negative	199 (59.4)	96 (72.7)	0.50 (0.16-1.5)
Positive	136 (40.6)	36 (27.3)	0.22 (0.10-0.54)
	$P = 0.0077$		
Syphilis			
Negative	321 (96.1)	135 (100)	0.34 (0.18–0.62)
Positive	13 (3.9)	0 (0)	–
	$P = 0.0024$		
Chlamydial infection			
Negative	340 (97.4)	132 (97.8)	0.28(0.15–0.54)
Positive	9 (2.6)	3 (2.2)	1.8 (0.10–31)
	$P = 1.0$		
Gonorrhoea infection			
Negative	348 (100)	135 (100)	–
Positive	0 (0)	0 (0)	–
Episode of sexually transmitted infection in past 12 months			
No	279 (77.3)	122 (86.5)	0.2 (0.10–0.47)
Yes	82 (22.7)	19 (13.5)	0.89 (0.31–2.6)
	$P = 0.025$		
Frequent condom use with non-spousal partners[b]			
No	141 (82.0)	60 (76.9)	0.33 (0.11–1.0)
Yes	31 (18.0)	18 (23.1)	0.65 (0.11–3.8)
	$P = 0.39$		

[a] Full time, full-time employed; other, includes self-employed, part-time or irregularly employed, looking for a job, being a homemaker.
[b] Frequent condom use, used a condom always or most of the time with all non-spousal partners of the past 12 months.

Table 4. Association between HIV infection and socio-demographic and behavioural risk factors and sexually transmitted infections among sexually active men in Kisumu: univariate analysis

	OR (95% confidence interval)			OR (95% confidence interval)	
	All men	Luo men only		All men	Luo men only
Age	$P < 0.001$	$P < 0.001$	Occupation	$P = 0.005$	$P = 0.002$
15–19 years	1	1	Full time	1	1
20–29 years	4.4 (1.7–11.5)	6.4 (2.2–19)	Student	0.10 (0.023–0.43)	0.082 (0.19–0.36)
30–39 years	9.4 (3.6–24.7)	13 (4.4–38)	Other	1.1 (0.68–1.7)	1.1 (0.62–1.8)
40–49 years	7.5 (2.7–21.2)	7.2 (2.3–23)			
			Ethnic group	$P = 0.0003$	NA
Marital status	$P < 0.001$	$P < 0.001$	Luo		
Never married	1	1	Other	3.5 (1.8-7.0)	
Ever married	5.8 (3.3–10.2)	5.9 (3.2–10.6)			
			Religion	$P = 0.41$	$P = 0.79$
Age at first sex	$P = 0.9$	$P = 0.65$	Christian	1	1
>14 years	1	1	Muslim	0.42 (0.10-1.9)	1.0 (0.10-9.7)
≤ 14 years	1.0 (0.65–1.6)	0.89 (0.65–1.6)	Other	1.2 (0.67-2.2)	1.1 (0.62-1.8)
Number lifetime partners	$P = 0.001$	$P = 0.004$	Travel in the past 12 months	$P = 0.37$	$P = 0.98$
1–3	1	1	< 2 trips	1	1
4–9	2.6 (1.4–4.7)	2.4 (1.2–4.5)	> 1 trip	0.82 (0.52–1.3)	1.0 (0.62–1.6)
> 9	3.2 (1.7–5.9)	3.0 (1.6–5.9)			
			Circumcision	$P = 0.0001$	$P = 0.02$
Number of non-spousal partners in past 12 months	$P = 0.0001$	$P = 0.0002$	No	1	1
			Yes	0.30 (0.17–0.55)	0.33 (0.13–0.86)
0	1	1			
1	0.46 (0.28–0.76)	0.43 (0.25–0.75)	Herpes simplex virus type 2	$P < 0.001$	$P < 0.001$
> 1	0.31 (0.17–0.59)	0.30 (0.15–0.60)	Negative	1	1
			Positive	9.7 (5.6–17)	11.1 (6.1–20)
One-off contact or commercial sex in past 12 months					
0	$P = 0.08$	$P = 0.14$	Syphilis	$P = 0.04$	$P = 0.09$
≥ 1	0.39 (0.10–1.1)	0.40 (0.12–1.4)	Negative	1	1
			Positive	3.2 (1.1-9.8)	2.6 (0.85-8.0)
Alcohol	$P < 0.001$	$P < 0.001$			
< Once a month	1	1	Chlamydial infection	$P = 0.76$	$P = 0.84$
≥ Once a month	2.5 (1.6–3.8)	2.8 (1.7–4.4)	Negative	1	1
			Positive	1.2 (0.33–4.6)	0.85 (0.17–4.2)
Education	$P = 0.64$	$P = 0.93$			
No primary	1	1	Episode of sexually transmitted disease in past 12 months	$P = 0.006$	$P = 0.03$
Primary complete	1.1 (0.67–1.9)	1.1 (0.61–1.9)	No	1	1
Secondary/higher	0.90 (0.51–1.6)	0.99 (0.53–1.8)	Yes	2.2 (1.2–3.7)	1.9 (1.1–3.5)

NA, Not applicable; OR, odds ratio.

Male circumcision and HIV in Ndola

In Ndola, there was little difference in the prevalence of HIV infection among men who were circumcised and men who were not (OR = 0.95, 95% CI = 0.46–1.94). There were no differences between both groups of men in terms of socio-economic characteristics and sexual behaviour (data not shown). In contrast to Kisumu, there was also no difference in prevalence of HSV-2 (42% in circumcised men and 40% in uncircumcised men), syphilis (11% in circumcised men and 13% in uncircumcised men), or in the proportion of men who reported a STI episode in the past 12 months (18 and 19%).

When looking at the association between circumcision and HIV infection by marital status, we found that none

Table 5. Multivariate model of the association between circumcision status and HIV among sexually active men in Kisumu

	Odds ratio (95% confidence interval)			
	All men		Luo men only	
	Model without variables related to STI	Model with variables related to STI	Model without variables related to STI	Model with variables related to STI
Circumcision	$P = 0.0000$	$P = 0.0006$	$P = 0.016$	$P = 0.020$
No	1	1	1	1
Yes	0.27 (0.15–0.51)	0.26 (0.12–0.56)	0.29 (0.11–0.79)	0.21 (0.06–0.78)
Marital status	$P = 0.0000$	NE	$P = 0.0000$	NE
Never married	1		1	
Ever married	5.3 (3.0–9.5)		5.1 (2.8–9.4)	
Alcohol	$P = 0.0019$	$P = 0.0021$	$P = 0.0012$	$P = 0.0005$
< Once a month	1	1	1	1
≥ Once a month	2.1 (1.3–3.3)	2.3 (1.4–3.9)	2.3 (1.4–3.8)	2.8 (1.6–5.0)
Episode of sexually transmitted disease in past year	NA	$P = 0.021$	NA	$P = 0.047$
No		1		1
Yes		2.2 (1.1–4.4)		2.2 (1.0–4.6)
Herpes simplex virus type 2	NA	$P = 0.0000$	NA	$P = 0.0000$
Negative		1		1
Positive		8.8 (5.0–16)		10.7 (5.7–20)

STI, Sexually transmitted infection; NE, not entered by the stepwise procedure; NA, not applicable.

Table 6. Comparison of reported characteristics (%) of partners of circumcised men and partners of men who were not circumcised, in Kisumu

	Partners of uncircumcised men ($n = 329$)	Partners of circumcised men ($n = 133$)
Age		
< 15 years	6.7	2.3
15–19 years	64.7	54.1
20–24 years	18.5	24.8
25–29 years	7.9	11.3
> 29 years	2.1	7.5
Marital status		
Never married	82.4	82.3
Now married	9.8	6.4
Past marriage	4.0	5.0
Do not know	3.7	3.7
Ethnic group		
Luo	88.2	58.9
Other	11.8	41.1
Estimated prevalence of HIV	32%	32%

of 18 circumcised, never-married men were HIV infected, whereas the prevalence of HIV infection among uncircumcised, never-married men was 12% (20/167). This difference was not statistically significant ($P = 0.23$). Among men who were married or had been married in the past, the prevalence of HIV infection was 42% (11/26) in circumcised men and 34% (96/282) in uncircumcised men. It seemed that circumcised men were more often married to an HIV-infected woman than uncircumcised men. Of the HIV-negative married men, 3/10 spouses (30%) of circumcised men were HIV infected, compared with 15/135 (11%) of uncircumcised men. Multivariate analysis was carried out on the pooled data of never-married men and men who were married to an HIV-uninfected woman. This gave an OR for HIV infection associated with circumcision of 0.5 (95% CI = 0.1–2.2). This OR was similar to that in Kisumu (OR = 0.3, 95% CI = 0.06–1.5).

Discussion

We found a strong protective effect of male circumcision in Kisumu, Kenya, with an OR of 0.2–0.3. In this city, circumcision is not traditionally practised by the main ethnic group, the Luo, whereas men belonging to other ethnic groups are mostly circumcised. The association

between circumcision and HIV infection persisted after allowing for possible confounding factors, including socio-economic factors, sexual behaviour and other STIs. In addition, we compared the prevalence of HIV infection in spousal and in non-spousal partners of circumcised men and uncircumcised men. According to our estimates, there was no difference in HIV prevalence among non-spousal partners of both groups of men. The data on spouses suggested that circumcised men may less likely be married to an HIV-infected woman, but the twofold difference in HIV prevalence of spouses of HIV-negative men could not explain the three to fivefold difference in odds of HIV infection associated with circumcision.

In Rakai Region, Uganda, the strongest protective effect of circumcision was found in men who were circumcised before age 13 [7]. However in Mwanza Region, Tanzania, the opposite was found, i.e. circumcision after age 14 was protective against HIV infection while circumcision before age 15 was associated with an increased risk of HIV infection [8]. In our study in Kisumu, men who were circumcised before they had their first sexual experience had a lower prevalence of HIV infection than men who were circumcised after they had become sexually active. However, the number of men who were circumcised after their sexual debut was too small to perform a separate multivariate risk factor analysis and to compare the odds ratio for HIV infection in this group with the odds ratio in men who were circumcised before age at first sex.

The prevalence of HSV-2 infection and of syphilis was significantly lower in circumcised men than in uncircumcised men in Kisumu. Analysis of risk factors for HSV-2 infection found circumcision to have a protective effect with an adjusted OR of 0.4 [9]. Indeed, one proposed mechanism to explain the protective effect of circumcision against HIV infection is its protective effect against other STIs, in particular ulcerative STIs [10]. However, when we added HSV-2 and history of STI in the past 12 months to the logistic regression model, the association between circumcision and HIV infection was not weakened as one would have expected if the effect of circumcision were mainly through other STIs. This suggests that, in Kisumu, the protective effect of circumcision is mainly a direct biological effect. Several mechanisms have been proposed to explain this including increased likelihood of abrasions in the presence of a foreskin and the presence of Langerhans cells in the foreskin [11,12].

The data from Ndola are more difficult to interpret. Circumcision is less common and power was limited, but there were some striking differences with Kisumu. In contrast to Kisumu, the prevalence of HSV-2 and of syphilis was the same in circumcised and in uncircumcised men in Ndola. This may suggest that circumcised men in Ndola were more exposed to infected partners. The data on the spouses go in the same direction as they suggest that circumcised men in Ndola were more often married to an HIV-infected woman. When restricting the analysis to men who were never married or men who were married to an HIV-uninfected woman, circumcision showed a protective effect, although it was not statistically significant.

In conclusion, our data on the association between male circumcision and HIV infection at the individual level add to the existing body of evidence for a protective effect of circumcision against the acquisition of HIV infection by men [5]. The main objective of the multicentre study was to try and identify factors that could explain the differences in rate of spread of HIV between different cities in sub-Saharan Africa. We found striking differences in the proportion of men who were circumcised between the low HIV prevalence cities, where almost all men were circumcised, and the high HIV prevalence cities, where the majority of men were uncircumcised. The difference between our study and earlier ecological studies was that we collected data on sexual behaviour and on other STIs in each of the four populations. We found important differences in sexual behaviour between the four cities but they could not by themselves explain the differences in rate of spread of HIV [13]. For instance, the rate of partner change was higher in Yaoundé, one of the low HIV prevalence cities, than in Kisumu and Ndola. We concluded that the differences in rate of spread of HIV were rather due to differences in probability of transmission of HIV during sexual intercourse, and two factors were identified that enhance the probability of transmission in Kisumu and Ndola, i.e. lack of male circumcision and HSV-2 infection [14].

There is considerable evidence that circumcision protects men against the acquisition of HIV infection [5,15] and it is time now to seriously consider male circumcision as a strategy to prevent the spread of HIV [16,17]. Several issues will need to be addressed when considering such intervention. First, the evidence we have so far comes from observational studies of the protective effect of circumcision at the individual level. We need studies on the effect of male circumcision at the population level. The magnitude of this effect is likely to differ from one population to another. Studies on circumcision as a risk factor for HIV infection have found variations in the strength of the association between male circumcision and HIV infection, which may be due to interaction with other STIs or with genital hygiene practices [18]. Second, there are concerns about decrease in safe sexual practices if circumcision is perceived to offer full protection against HIV infection. Finally, there are issues of acceptability, feasibility, cost-effectiveness, safety and evaluation of the intervention. A recent feasibility study found that male circumcision in Kisumu may be accept-

able by non-circumcising populations if presented as a measure to improve genital hygiene [19]. More research of this type is needed, as well as research on how best to promote circumcision. The risks and benefits of promotion of male circumcision will have to be weighed against each other, but we cannot continue to ignore a potentially very effective intervention against HIV.

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Sexual behaviour patterns and other risk factors for HIV infection in rural Tanzania: a case–control study

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Objective: To examine the association between HIV infection and patterns of sexual behaviour and other risk factors in a rural Tanzanian population in a case–control study, nested within a randomized trial of improved sexually transmitted disease treatment.

Methods: All HIV-positive patients from the baseline survey of the randomized trial were eligible as cases. Cases (n = 338) and controls (a random sample of one in eight HIV-negative persons; n = 1078) were interviewed about risk factors for HIV infection using a structured questionnaire.

Results: A significantly higher HIV prevalence was found among men and women not currently employed in farming [men: odds ratio (OR), 2.08; women: OR, 3.65], women who had travelled (OR, 3.27), educated women (OR, 4.51), and widowed/divorced people compared with those currently married (men: OR, 3.10; women: OR, 3.54). Two spouse-related factors were significantly associated with HIV, even after adjustment for the sexual behaviour of the index case: HIV was more prevalent in men with younger spouses ($P = 0.020$ for trend) and in women married to men currently employed in manual work, office work or business (OR, 2.20). In women only, blood transfusions were associated with a higher HIV prevalence (OR, 2.40), but only a small population attributable fraction (4%). There was an increased HIV prevalence associated with increasing numbers of injections. Reported number of lifetime sexual partners was significantly associated with HIV infection (women: OR, 7.33 if ≥10 lifetime partners compared with ≤1; men: OR, 4.35 for ≥50 compared with ≤1). After adjustment for confounders, male circumcision was associated with a lower HIV prevalence (OR, 0.65; $P = 0.11$).

Conclusions: In these rural communities, many HIV infections occur through sexual transmission. Some people are at high risk of HIV infection through large numbers of sex partners, whereas some are at risk through their spouse or regular partner. The role of circumcision in HIV transmission is unclear. Commercial sex seems to play a negligible role in HIV transmission in these communities. Our results confirm marked heterogeneity in HIV risk, indicating the scope for risk reduction strategies.

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Keywords: HIV-1, rural population, Africa, risk factors, sexual behaviour, case–control study

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Introduction

AIDS has now been documented as the leading cause of adult death in Côte d'Ivoire, Zaïre and Uganda [1], and HIV-related deaths have substantially increased mortality rates in many parts of sub-Saharan Africa. The HIV epidemic in sub-Saharan Africa is spread predominantly through heterosexual contact [2]. Groups that are at particularly high risk of HIV infection have been identified as commercial sex workers, those attending sexually transmitted disease (STD) clinics, and truck drivers [3].

The transmission dynamics of HIV infection outside these high-risk groups are not clear. Studies that have been conducted in rural African populations have identified sociodemographic risk factors for HIV infection [4–12]. This information is useful for the planning of HIV intervention programmes, but additional information on sexual attitudes and behaviour is needed to design appropriate educational campaigns. Most previous studies [4–8,10–12] have obtained some information on sexual behaviour: multiple sex partners, history of sex with commercial sex workers, and history of STD were identified as risk factors for HIV. However, no studies to date have sought detailed information on types of sexual partnerships or patterns of casual sex in such populations. Such information might be used to focus HIV interventions on those at greatest risk of infection.

In 1991, implementation of an STD intervention programme began in 12 rural communities of Mwanza Region, Tanzania. The impact of this intervention programme was assessed in a community randomized trial [13–15]. Patients with HIV infection and other STD at baseline were identified from the cohort of subjects participating in the trial. These individuals, together with a random subsample of the cohort, were selected for detailed questioning about sexual attitudes and practices. The random subsample formed the basis for a survey of sexual behaviour in this population. In addition, case–control analyses were carried out by comparing cases of HIV or STD with controls drawn appropriately from the random subsample. The results of the sexual behaviour survey and the nested case–control study for STD will be reported separately. We report here the results of a case–control study of the association of HIV prevalence with sexual behaviour patterns and other risk factors in this rural Tanzanian population.

Methods

The demographic characteristics of the study population have been described previously [13]. Implementation of a programme of improved STD treatment services began in the Mwanza Region in December 1991. A baseline survey was conducted prior to implementation and has been described previously [14]. In brief, a cohort of approximately 1000 adults aged 15–54 years was selected from each of 12 rural communities using random cluster sampling. This cohort of 12 537 adults was enrolled, interviewed and examined between November 1991 and December 1992, prior to implementation of the intervention in each community. A sample of venous blood was taken from consenting adults and sera were tested for HIV antibodies by enzyme-linked immunosorbent assay (ELISA; Vironostika HIV MIXT Microelisa, Organon Technika, Boxtel, The Netherlands). All positive samples underwent confirmatory testing with a methodologically independent ELISA (Wellcozyme HIV 1+2 GACELISA, Murex Diagnostics, Dartford, Kent, UK). In the case of discrepant or indeterminate ELISA results, a confirmatory Western blot was performed (HIV-1 Westernblot, Epitope, Beaverton, Oregon, USA). Individuals wishing to know their HIV test result were referred for pre- and post-test counselling. HIV tests were performed on 12 500 (99.7%) of those enrolled and these individuals formed the sampling frame for a nested case–control study of HIV infection. Overall, 219 men (3.7% of the total) and 291 women (4.4%) were HIV-positive, and were therefore eligible for selection as cases. The controls were drawn from a simple random sample of one in eight persons in the sampling frame. All HIV-negative persons in this random sample were eligible as controls.

An attempt was made to revisit all eligible cases and controls. After obtaining informed consent, cases and controls were interviewed about sexual and non-sexual risk factors. A structured questionnaire was used to ascertain sociodemographic details and information on blood transfusions, injections, sexual attitudes, sexual practices, and perception of risk. In the questionnaire, sexual partners were classified as 'marital' (spouses/constant partners), 'regular' (partners for more than a few weeks), or 'casual' (partners for no more than a few weeks, including 'one-off' partners and commercial sex workers). Men and women were asked whether they ever got bruises on their sexual organs because of sexual intercourse. Women were asked whether they ever practised 'dry sex', involving the insertion of herbs or other substances into the vagina to reduce vaginal secretions. The questionnaire was designed in Kiswahili, back-translated into English and pre-tested in a pilot study in February 1993. Non-medical personnel, most of whom were teachers, community development workers or cultural officers from Mwanza Region were carefully selected and trained in interview techniques. Interviewers were unaware of the participants' HIV status. Very few participants requested their HIV test result and most were unaware of their HIV status.

The study took place in May and June 1993, 6–17 months after the baseline survey of the 12 study communities.

In the analysis, each sex was considered separately. Risk factors for HIV have been broadly categorized as either sociodemographic or 'proximate' factors, the latter including factors likely to be directly related to HIV transmission. Odds ratios (OR), adjusted for age-group (15–19, 20–24, 25–29, 30–34, 35–44 and 45–54 years) and community of residence, were obtained for all risk factors using logistic regression. For most of the sociodemographic factors, no adjustment was made for additional confounders, because the aim was to describe the relative risk in different classes of individual, rather than to isolate the specific effect of a particular variable. OR for proximate factors and those sociodemographic factors relating to an individual's spouse were adjusted for confounders. All risk factors found to be significant after adjusting for age-group and community of residence were fitted in a logistic model. A final model included only those factors which remained significant after adjusting for all other factors in the model. Significance was assessed using the likelihood ratio test. Logistic regression was performed in Egret (Statistics and Epidemiology Research Corporation, Seattle, Washington, USA). Adjusted OR were used to estimate the population attributable fraction for each exposure [16]. Each population-attributable fraction estimates the proportion of HIV infections that would have been prevented had the risk for all subjects been as low as in the baseline category while all other factors were kept constant. Although each population-attributable fraction is adjusted for confounders, it is not possible to estimate the joint population-attributable fraction for several exposures by summing several population-attributable fraction.

Results

The participation rate among those sought for this case–control study was 66% for cases and 75% for controls (Table 1). Non-participation was largely due to the person not being found (20% had moved, travelled, died or could not be traced). Only 0.5% of those found refused to be interviewed. Fifty-four persons were excluded because of doubts about identity. The age distribution of cases and controls is also shown in Table 1. Since no male cases were under 20 years of age, the remainder of the analysis of men was restricted to the 149 cases and 394 controls aged 20–54 years. The analysis of women was conducted on all 189 cases and 574 controls.

Sociodemographic risk factors in men and women

OR for sociodemographic risk factors are shown in Table 2. There was no significant association between education and HIV infection in men. In women, the prevalence of HIV infection increased significantly with level of education, the small group of women with secondary education or higher having four times the prevalence of those with no education. Men and women in manual work, office work or business had a significantly higher prevalence of HIV infection than farmers, as did men in 'other' occupations (mostly fishermen). Those who had lived elsewhere in the past 5 years had a higher prevalence of HIV infection, especially men who had lived in another large town [OR, 2.13; 95% confidence interval (CI), 1.11–4.07], women who had lived in Mwanza town (OR, 3.52; 95% CI, 1.62–6.27), and women who had lived in another large town (OR, 3.19; 95% CI, 1.62–6.27). Travel was much less common among women than men but was more strongly associated with HIV. In particular, travel to another large town in the past year was significantly associated with HIV infection in women and gave rise to a population-attributable fraction of 9.7%. The prevalence of HIV infection was somewhat higher among Moslems, although in men this was not statistically significant. Moslems in this study shared a number of characteristics, some of which may have put them at higher risk of infection. Among the controls, Moslem women were more likely than other women to have lived in a large town in the past 5 years (14 versus 3%; $P = 0.011$) and to report five or more lifetime partners (30 versus 18%; $P = 0.13$), but their spouses were not

Table 1. Participation rates and reasons for non-recruitment among HIV-positive cases and HIV-negative controls, by sex.

	Men		Women	
	Cases	Controls	Cases	Controls
Sought (n)	219	659	291	781
Participated (n)	149	504	189	574
Participation rate (%)	68	76	65	73
No. participated, by age-group (years)				
15–19	0	110	13	96
20–24	22	82	57	108
25–29	24	60	39	84
30–34	38	78	33	85
35–44	38	75	30	107
45–54	27	99	17	94
Total	149	504	189	574
Reasons for non-recruitment (n)				
Died*	9	1	11	5
Moved*	26	48	37	87
Refused	0	1	1	7
Travelled	13	47	13	33
Not traced†	8	15	15	23
Other	8	26	19	27
Excluded‡	6	17	6	25
Total	70	155	102	207

*Since the baseline survey. †Person unknown and unable to be traced within the community. ‡Individuals were excluded if at the time of interview their identity with the person originally enrolled in the baseline survey was doubted.

Table 2. Odds ratios (OR) for selected sociodemographic factors, by sex.

Variables	Men				Women			
	No. cases	No. controls	OR* (95% CI)	$P(P_T)$	No. cases	No. controls	OR* (95% CI)	$P(P_T)$
Education								
None/adult	20	82	1	0.39 (0.17)	49	263	1	0.004 (<0.001)
Primary 1–3	24	66	1.72 (0.84–3.55)		24	51	1.97 (1.07–3.65)	
Primary ≥4	93	227	1.62 (0.86–3.06)		110	255	2.08 (1.32–3.28)	
Secondary/higher	12	19	1.77 (0.70–4.50)		6	5	4.51 (1.20–16.93)	
Present job								
Farmer	101	331	1	0.002	164	546	1	0.005
Manual/office/business	35	45	2.08 (1.21–3.60)		19	13	3.65 (1.66–7.99)	
Other	13†	18†	3.08 (1.32–7.15)		2‡	13‡	1.07 (0.21–5.45)	
Lived elsewhere (past 5 years)								
No	91	294	1	0.011	117	432	1	0.022
Yes	58	99	1.76 (1.14–2.71)		72	141	1.57 (1.07–2.31)	
Travel to Mwanza (past year)								
No	78	246	1	0.41	129	472	1	0.012
Yes	71	148	1.20 (0.77–1.87)		59	102	1.72 (1.13–2.62)	
Travel to other large town (past year)								
No	123	335	1	0.98	160	548	1	<0.001
Yes	26	59	0.99 (0.57–1.72)		26	26	3.27 (1.78–6.03)	
Ethnic group								
Sukuma	109	254	1	0.50	130	396	1	0.248
Mkara	7	55	0.71 (0.13–3.91)		10	59	1.60 (0.50–5.13)	
Other	33	83	1.32 (0.77–2.27)		49	118	1.50 (0.93–2.41)	
Religion								
Moslem	12	14	1	0.11	20	28	1	<0.001
Catholic	68	151	0.64 (0.26–1.55)		93	228	0.55 (0.28–1.07)	
Protestant	41	105	0.46 (0.18–1.16)		55	150	0.45 (0.22–0.91)	
Other	28	124	0.38 (0.15–0.99)		21	167	0.22 (0.10–0.49)	
Marital status								
Currently married	107	325	1	0.008	131	462	1	<0.001
Divorced/widowed	21	18	3.10 (1.52–6.34)		38	48	3.54 (2.07–6.04)	
Never married	15	42	1.07 (0.51–2.26)		12	53	1.30 (0.57–3.00)	
Previous marriage that ended (if married)								
No	51	202	1	0.012	74	306	1	<0.001
Yes	48	106	1.92 (1.15–3.21)		36	117	1.66 (0.99–2.77)	
Age of current spouse (years)								
<20	11	39	1	0.096 (0.014)	13	32	1	0.991
20–24	28	70	0.81 (0.32–2.04)		37	96	0.95 (0.42–2.13)	
25–34	42	107	0.58 (0.22–1.56)		27	79	1.14 (0.44–2.89)	
35–44	13	62	0.33 (0.10–1.12)		15	70	1.05 (0.35–3.14)	
≥45	1	20	0.08 (0.01–0.81)		6	27	1.17 (0.29–4.71)	
Spouse divorced/widowed								
No	60	215	1	0.085	61	279	1	0.012
Yes	39	90	1.59 (0.94–2.68)		49	144	1.82 (1.14–2.88)	
No. wives§								
1	87	277	1	0.26	90	346	1	0.167
≥2	20	47	1.45 (0.77–2.73)		40	113	1.39 (0.87–2.22)	

*Adjusted for age-group and community. †Mostly fishermen. ‡Mostly students/school pupils. §For women, number of wives of current spouse. P_T, test for trend P value; CI, confidence interval.

more likely to have more than one wife (18 versus 25%; $P = 0.47$). Moslem men were more likely than other men to be circumcised (79 versus 29%; $P < 0.001$), to have lived in roadside settlements in the past 5 years (36 versus 7%; $P = 0.003$), to have travelled to Mwanza in the past year (64 versus 37%; $P = 0.036$), to have secondary or higher education (14 versus 5%; $P = 0.054$), and to report 10 or more lifetime partners (79 versus 56%; $P = 0.096$), but were not significantly more likely to have more than one wife (20 versus 14%; $P = 0.62$).

Current and past marital status was associated with HIV infection (Table 2). Men and women who were divorced or widowed were three times more likely to be HIV-positive than those currently married, and in women the HIV prevalence was higher in those never married than in those currently married. The higher prevalence in divorced or widowed women than among currently married women may be due partly to a greater number of lifetime partners and casual partners (28 versus 19% reported five or more lifetime partners, and 28 versus 7% reported a casual partner in the past year). Men who were divorced or widowed reported similar numbers of lifetime partners to married men, but more casual partners in the past year. For example, 33% of divorced or widowed men and 11% of those currently married reported three or more casual partners in the past year. Among those currently married, a substantial proportion (34% of male controls, 28% of female controls) had previous marriages that ended in

Table 3. Odds ratios (OR) for marital status shown within different age-groups, by sex.

Marital status	Men			Women		
	No. cases	No. controls	OR* (95% CI)	No. cases	No. controls	OR* (95% CI)
Age 20–29 years						
Currently married	30	93	1			
Divorced/widowed	4	6	1.56 (0.34–7.18)			
Never married	7	37	0.48 (0.17–1.31)			
Age 15–29 years				81	224	1
Currently married				12	8	3.70 (1.31–10.46)
Divorced/widowed				11	50	1.35 (0.55–3.31)
Never married						
Age 30–54 years						
Currently married	77	232	1	50	238	1
Divorced/widowed	17	12	3.81 (1.64–8.82)	26	40	2.74 (1.34–5.61)
Never married	8	5	4.31 (1.20–15.52)	1	3	4.37 (0.35–55.03)

*Adjusted for age-group and community. CI, confidence interval.

divorce or widowhood, and these individuals had a higher HIV prevalence. There was no association between number of wives and HIV prevalence.

OR for marital status within different age-groups are shown in Table 3. In men, there was a significant interaction between marital status and age ($P = 0.017$). In men aged 30–54 years, a fourfold increased prevalence was found in those never married and in those divorced or widowed, compared with those currently married. In men aged 20–29 years, there was a higher prevalence in those divorced or widowed, but the group of men who had never married had only half the prevalence of those currently married. There was no interaction between marital status and age in women ($P = 0.44$), although, as for men, the highest prevalence was found among those aged 30–54 years who had never married.

Analysis of the spouse-related factors suggested that some married men and women may be at increased risk of HIV infection through their spouses (Table 2). First, among men there was a significant trend with age of current spouse (the younger the spouse, the higher the prevalence of HIV infection), which remained significant after adjustment for confounders ($P = 0.020$). Second, those men whose spouses had previously been widowed or divorced had an increased HIV prevalence although after adjustment for confounders, this excess was not statistically significant ($P = 0.145$). Among married women, there was no association between age of current spouse and HIV infection. However, those women whose spouse had previously been widowed or divorced had a higher HIV prevalence, although this effect was smaller and not significant after adjustment for confounders (OR, 1.46; 95% CI, 0.89–2.42). Furthermore, those women married to men employed in manual work, office work or business had a twofold increased HIV prevalence, even after adjustment for confounders (OR, 2.20; 95% CI, 1.22–3.95).

Proximate risk factors for men

OR for proximate risk factors among men are shown in Table 4. Only 2% of cases and controls had received a blood transfusion during the past 5 years; therefore, transfusions are clearly not a major source of infection in this population. There was a significant upward trend between HIV infection and the number of injections received in the past year, although this trend was no longer significant after adjustment for confounders. A substantial proportion of men (62% of controls) reported skin incisions or tattoos, but these men did not have a higher prevalence of HIV.

Most men (63% of cases, 65% of controls) had their first sexual intercourse aged 15–19 years, and 90% of men had their first sexual intercourse before they were aged 20 years. Age at first sexual intercourse was similar in cases (mean, 15.9 years) and controls (mean, 16.1 years) and was not associated with HIV infection ($P = 0.90$). There was a very wide variation in the reported number of lifetime partners, with 34% of controls reporting 20 or more partners and 18% reporting fewer than five. All men reported at least one sex partner. There was a highly significant trend for increasing HIV prevalence with increasing partners ($P = 0.002$), but the trend weakened after adjustment for confounders ($P = 0.059$). The population-attributable fraction for reporting more than one lifetime sex partner was 66% and the population-attributable fraction for reporting more than four lifetime sex partners was 18%.

We examined the association between HIV infection and reported number of casual and non-casual partners in the past year. Before adjustment for confounders, there was no association between the number of sexual partners in the past year and HIV infection. After adjustment for confounders, there was a significant negative trend ($P = 0.011$), those men reporting no partners having the highest prevalence and those reporting five or more partners having the lowest prevalence. A strong negative trend was also observed between the number of casual partners in the past year and HIV infection. Since the number of sexual partners in the past year was strongly correlated with lifetime

Table 4. Odds ratios (OR) for selected proximate factors in men aged 20–54 years.

Variables	n (%) Cases	Controls	OR* (95% CI)	$P(P_T)$	OR† (95% CI)	$P(P_T)$
Blood transfusion (past 5 years)						
No	146 (98)	387 (98)	1	0.96	1	0.60
Yes	3 (2)	7 (2)	0.97 (0.23–4.11)		0.59 (0.08–4.61)	
Injections (past year)						
None	51 (34)	204 (52)	1	0.049 (0.010)	1	0.27 (0.12)
1	9 (6)	29 (7)	1.27 (0.54–2.97)		1.34 (0.50–3.57)	
2–4	37 (25)	59 (15)	2.14 (1.24–3.69)		2.20 (1.10–3.70)	
5–9	29 (20)	62 (16)	1.77 (0.99–3.15)		1.32 (0.69–2.54)	
≥10	23 (15)	39 (10)	1.82 (0.96–3.46)		1.42 (0.67–3.00)	
Skin incision/tattoos						
No	61 (41)	150 (38)	1	0.98	1	0.76
Yes	87 (59)	242 (62)	0.99 (0.66–1.50)		0.93 (0.58–1.49)	
Lifetime sex partners						
0–1	1 (1)	12 (3)	0.37 (0.04–3.24)		0.40 (0.04–3.86)	
2–4	16 (11)	59 (15)	1	0.036 (0.002)	1	0.48 (0.059)
5–9	27 (19)	96 (25)	1.13 (0.54–2.39)		0.89 (0.39–2.01)	
10–19	30 (21)	90 (23)	1.27 (0.61–2.64)		1.09 (0.49–2.44)	
20–49	38 (27)	85 (22)	1.58 (0.75–3.31)		1.33 (0.59–2.98)	
≥50	31 (22)	46 (12)	2.99 (1.32–6.78)		1.74 (0.71–4.26)	
Sex partners (past year)						
None	14 (9)	15 (4)	3.08 (1.32–7.18)		3.45 (1.08–11.00)	
1	58 (39)	168 (43)	1	0.13 (0.29)	1	0.059 (0.011)
2	30 (20)	86 (22)	1.07 (0.62–1.85)		0.84 (0.45–1.56)	
3–4	31 (21)	84 (21)	0.98 (0.57–1.70)		0.66 (0.35–1.26)	
≥5	16 (11)	41 (10)	1.04 (0.51–2.13)		0.50 (0.21–1.18)	
Casual partners (past year)						
None	86 (58)	226 (57)	1	0.93 (0.56)	1	0.048 (0.002)
1	26 (17)	75 (19)	0.84 (0.49–1.44)		0.66 (0.35–1.23)	
2	17 (11)	38 (10)	0.99 (0.50–1.94)		0.50 (0.23–1.09)	
3–4	13 (9)	36 (9)	0.76 (0.36–1.58)		0.40 (0.17–0.97)	
≥5	7 (5)	19 (5)	0.91 (0.34–2.41)		0.24 (0.07–0.80)	
Commercial sex workers (past year)						
None	143 (96)	386 (98)	1	0.25	1	0.69
≥1	6 (4)	8 (2)	1.95 (0.64–5.88)		1.27 (0.39–4.14)	
Bruising during sex						
No	91 (62)	295 (75)	1	0.046	1	0.80
Yes	56 (38)	96 (25)	1.60 (1.01–2.54)		1.07 (0.64–1.79)	
Ever used condom						
No	107 (72)	314 (80)	1	0.15	1	0.37
Yes	42 (28)	78 (20)	1.44 (0.88–2.34)		0.77 (0.43–1.37)	
Genital ulcer/discharge (past year)						
No	95 (66)	308 (78)	1	<0.001	1	0.037
Yes	50 (34)	85 (22)	2.22 (1.40–3.52)		1.77 (1.04–3.02)	
Circumcised						
No	101 (68)	272 (70)	1	0.98	1	0.11
Yes	48 (32)	121 (31)	1.01 (0.64–1.58)		0.65 (0.38–1.12)	
Perceived risk of AIDS						
None/slight	30 (20)	104 (26)	1	0.43	1	0.52
Quite likely	90 (60)	220 (56)	1.22 (0.73–2.02)		0.70 (0.36–1.36)	
Very likely/infected	12 (8)	17 (4)	2.14 (0.87–5.26)		1.27 (0.45–3.61)	
Don't know	17 (11)	53 (14)	1.21 (0.58–2.49)		0.71 (0.27–1.84)	
Perceived risk STD						
None/slight	29 (20)	119 (30)	1	0.009	1	0.097
Quite likely	75 (50)	175 (44)	1.77 (1.05–2.99)		1.76 (0.97–3.18)	
Very likely/infected	35 (24)	69 (18)	2.86 (1.54–5.34)		2.46 (1.19–5.09)	
Don't know	10 (7)	31 (8)	1.90 (0.79–4.58)		1.74 (0.65–4.71)	
Known anyone with AIDS						
No	74 (50)	207 (52)	1	0.43	1	0.86
Yes	75 (50)	187 (47)	1.18 (0.78–1.78)		0.96 (0.60–1.53)	
How faithful is spouse						
Very faithful	19 (18)	84 (25)	1	0.027	1	0.260
Quite faithful	51 (48)	162 (50)	1.74 (0.92–3.31)		1.61 (0.81–3.19)	
Not faithful	8 (7)	17 (5)	3.16 (1.02–9.78)		1.83 (0.53–6.34)	
Don't know	29 (27)	61 (19)	2.84 (1.35–5.99)		2.21 (1.00–4.90)	

*Adjusted for age-group and community. †Adjusted for age-group, community, job, marital status, lifetime sex partners, genital ulcer/discharge, perceived risk of sexually transmitted disease (STD); the second lowest exposure group was chosen as the baseline category, if there were small numbers in the lowest exposure group. P_T, test for trend P value. CI, confidence interval.

partners, these effects were also examined without adjustment for lifetime partners. However, similar negative trends were observed even after adjustment for age, community, marital status, occupation, history of STD, and perceived risk of STD (data not shown). Those reporting sexual contact with commercial sex workers had a higher HIV prevalence, although numbers reporting such contacts were very small (4% of cases, 2% of controls) and the association was not significant. Casual sex with other types of partner was more common (in controls, 38% reported short-term partners and 9% reported other casual partners) but was not associated with HIV infection. Sex during travel away from home, or at dances, weddings or other traditional events is assumed to contribute to the risk of HIV infection. In controls, 13% reported casual sex during travel and 18% reported casual sex at dances or other events over the past year. However, neither of these were associated with HIV (OR, 0.70; 95% CI, 0.35–1.38 for travel; OR, 0.73; 95% CI, 0.40–1.31 for dances and other events). Bruising during sex was reported by 25% of controls and 38% of cases, but after adjustment for confounders, this effect was very weak (OR, 1.07) and not significant ($P = 0.80$). The main confounders were lifetime sex partners, history of genital ulcer or discharge, and perceived risk of STD.

There was no significant association between reported condom use and HIV infection, either before or after adjustment for confounders. Those reporting a history of genital ulcer or discharge during the past year had a higher HIV prevalence, even after allowing for confounders; the estimated fraction of HIV infections attributable to this effect was 15%.

The effect of circumcision on HIV prevalence was examined in a number of ways. Comparing circumcised men with non-circumcised men, and adjusting for confounders, circumcision showed a protective effect, although this was not statistically significant (Table 4). The main confounder of the effect of circumcision was occupation: 64% of non-farmers and only 26% of farmers were circumcised. Comparing men who had been circumcised before and after 15 years of age with non-circumcised men, and adjusting for confounders, showed a significant ($P = 0.027$) association with HIV: circumcision after age 15 years was associated with a lower HIV prevalence (OR, 0.48; 95% CI, 0.25–0.90), but circumcision before age 15 years was associated with a higher prevalence (OR, 1.34; 95% CI, 0.64–3.00). Circumcision was strongly associated with religion: among the controls, 64% of Moslems and 5% of non-Moslems were circumcised at birth, whereas 21% of Moslems and 71% of non-Moslems were never circumcised. However, excluding Moslems made little difference to the significance of the association with HIV ($P = 0.005$) or to the OR: after adjusting for confounders, circumcision after age 15 years was associated with a lower HIV prevalence (OR, 0.37; 95% CI, 0.18–0.74), but circumcision before age 15 years resulted in a higher prevalence (OR, 1.50; 95% CI, 0.57–3.90). The numbers were too small ($n = 26$) to examine the effect of circumcision in the Moslems.

The perception of being at risk of STD was significantly associated with HIV prevalence, but not the perception of being at risk of AIDS. Perceived risk of AIDS was associated with perceived risk of STD, but although men admitted to a high risk of STD (18% of controls), few men (4% of controls) would admit to being at high risk of AIDS. There was no association between knowing anyone with AIDS and HIV prevalence. Perceived faithfulness of spouse was significantly associated with HIV, those men reporting that their spouse was not faithful having the highest prevalence, although this effect was reduced after adjustment for confounders.

Proximate risk factors for women

OR for proximate risk factors among women are shown in Table 5. More women (4% of controls) than men had received a blood transfusion in the past 5 years, and this was associated with a twofold increased prevalence of HIV infection (OR, 2.40), but only a small population-attributable fraction (4%). There was a significant upward trend in HIV prevalence with increased number of injections, even after adjustment for confounders. Skin incisions or tattoos were associated with an increased HIV prevalence, although this effect was not significant after adjustment for confounders.

Most women (64% of cases, 66% of controls) had their first sexual intercourse aged 15–19 years, and almost all (92% of cases, 89% of controls) had their first sexual intercourse before they were 20 years. Age at first sexual intercourse was not associated with HIV infection ($P = 0.79$). The number of lifetime partners reported by women was lower and less varied than for men. However, most women reported more than one partner and a substantial proportion (18% of controls) reported at least five lifetime partners. Increasing lifetime partners were significantly associated with increasing HIV prevalence, even after adjustment for confounders ($P < 0.001$ for trend), with a sevenfold (3.59/0.49) increased prevalence in those women reporting 10 or more lifetime partners. The population-attributable fraction for reporting more than one lifetime partner was 50%, and the population-attributable fraction for reporting more than four lifetime partners was 17%. Only one case and 16 controls reported no sex partners ever. This case had received a blood transfusion in the past 5 years, but had received no injections in the past year and had no skin incisions or tattoos. It is likely that the blood transfusion was the mode of HIV transmission in this case.

Table 5. Odds ratios (OR) for selected proximate factors in women and aged 15–54 years.

Variables	n (%) Cases	Controls	OR* (95% CI)	$P(P_T)$	OR †(95% CI)	$P(P_T)$
Blood transfusion (past 5 years)						
No	175 (93)	553 (96)	1	0.079	1	0.039
Yes	14 (7)	20 (4)	1.98 (0.94–4.18)		2.40 (1.07–5.41)	
Injections (past year)						
None	68 (36)	278 (48)	1	0.006 (<0.001)	1	0.054 (0.006)
1	15 (8)	53 (9)	1.33 (0.67–2.64)		1.27 (0.58–2.77)	
2–4	41 (22)	124 (22)	1.12 (0.70–1.79)		1.12 (0.67–1.88)	
5–9	41 (22)	83 (14)	1.89 (1.16–3.09)		1.84 (1.07–3.17)	
≥10	23 (12)	35 (6)	2.92 (1.54–5.52)		2.55 (1.24–5.28)	
Skin incision/tattoos						
No	75 (40)	267 (47)	1	0.033	1	0.148
Yes	114 (60)	306 (53)	1.48 (1.03–2.13)		1.34 (0.90–2.01)	
Lifetime sex partners						
0–1	27 (15)	173 (31)	0.49 (0.29–0.81)		0.49 (0.28–0.84)	
2–4	92 (50)	277 (50)	1	<0.001 (<0.001)	1	<0.001 (<0.001)
5–9	40 (22)	83 (15)	1.27 (0.79–2.05)		1.24 (0.75–2.05)	
≥10	24 (13)	19 (3)	4.72 (2.33–9.54)		3.59 (1.65–7.78)	
Sex partners (past year)						
None	18 (10)	56 (10)	1.55 (0.82–2.94)		0.71 (0.30–1.67)	
1	137 (73)	461 (80)	1	0.010	1	0.430 (0.407)
2	21 (11)	42 (7)	1.96 (1.07–3.59)		1.02 (0.50–2.07)	
≥3	12 (6)	14 (2)	3.15 (1.32–7.50)		2.01 (0.78–5.20)	
Casual partners (past year)						
None	154 (82)	511 (89)	1	0.002 (<0.001)	1	0.658 (0.361)
1	25 (13)	49 (8)	2.48 (1.38–4.43)		1.21 (0.61–2.40)	
≥2	10 (5)	14 (2)	2.62 (1.05–6.54)		1.54 (0.54–4.44)	
Bruising during sex						
No	157 (85)	485 (90)	1	0.18	1	0.49
Yes	28 (15)	56 (10)	1.44 (0.85–2.43)		1.23 (0.68–2.23)	
Ever used condom						
No	180 (96)	558 (97)	1	0.830	1	0.112
Yes	7 (4)	16 (3)	1.12 (0.42–2.93)		0.38 (0.11–1.32)	
Genital ulcer/discharge (past year)						
No	147 (78)	482 (84)	1	0.014	1	0.194
Yes	42 (22)	90 (16)	1.76 (1.13–2.74)		1.41 (0.84–2.35)	
Perceived risk of STD						
None/slight	51 (27)	157 (27)	1	0.118	1	0.55
Quite likely	93 (49)	245 (43)	1.06 (0.69–1.63)		1.00 (0.62–1.61)	
Very likely/infected	23 (12)	51 (9)	1.53 (0.80–2.90)		1.40 (0.68–2.92)	
Don't know	22 (12)	121 (21)	0.65 (0.36–1.17)		0.77 (0.40–1.48)	
Perceived risk of AIDS						
None/slight	35 (18)	133 (23)	1	0.10	1	0.64
Quite likely	116 (61)	279 (49)	1.35 (0.84–2.15)		1.13 (0.67–1.90)	
Very likely/infected	8 (4)	15 (3)	1.95 (0.72–5.25)		1.24 (0.40–3.84)	
Don't know	30 (16)	147 (26)	0.81 (0.45–1.45)		0.81 (0.43–1.52)	
Known anyone with AIDS						
No	96 (51)	385 (67)	1	0.002	1	0.165
Yes	93 (49)	189 (33)	1.79 (1.25–2.56)		1.34 (0.89–2.01)	
How faithful is spouse						
Very faithful	12 (9)	71 (16)	1	0.34	1	0.24
Quite faithful	60 (46)	187 (41)	1.83 (0.89–3.75)		1.77 (0.84–3.75)	
Not faithful	28 (21)	102 (22)	1.54 (0.70–3.35)		1.18 (0.52–2.71)	
Don't know	31 (24)	99 (22)	1.88 (0.86–4.14)		1.86 (0.82–4.24)	

*Adjusted for age-group and community. †Adjusted for age-group, community, travel to another large town, religion, marital status and lifetime sex partners; baseline categories for lifetime sex partners and sex partners (past year) are consistent with those used in Table 4. P_T, test for trend P value; CI, confidence interval.

The few women who reported more than one sexual partner in the past year (9% of controls) or any casual partner in the past year (11% of controls) had a much higher HIV prevalence. These effects remained even after adjustment for age-group, community, travel, religion and marital status (data not shown). However, when further adjustment was made for lifetime partners, these effects were weaker and no longer significant (Table 5). Few women reported that they had casual sex during travel away from home (3% of controls), or at dances, weddings or other traditional events (4% of controls), and this was not associated with HIV prevalence. Bruising during sex was reported by 10% of controls and was associated with a small and non-significant increased HIV prevalence. Only 1% of women reported the practice of 'dry sex' ever, which was too few to examine its association with HIV.

Fewer women than men reported that they had ever used a condom and no association was found between condom use and HIV prevalence. Fewer women than men reported an STD in the past year and the association with HIV was not significant after adjustment for confounders. Very few women placed themselves in the category of being at high risk of STD (9% of controls) or AIDS (3% of controls). Risk perception was not significantly associated with HIV infection. However, there was a higher HIV prevalence among those women who had known anyone with AIDS, but this effect was weaker and not significant after adjustment for confounders. More women than men stated that their spouses were definitely 'not faithful' (22% compared with 5%), but this was not associated with HIV prevalence.

Discussion

Compared with most previous studies of risk factors for HIV infection in representative samples of rural African populations [4–12], our study has provided more detailed information on casual and non-casual sexual behaviour patterns. Controls were drawn from the same population as cases and were not matched on age or any other factor. The unmatched design employed here has several advantages. First, the same controls were used for case–control studies of several outcomes (HIV, syphilis and other STD), so for given resources each study yielded a greater power. Since cases and controls were not matched on community of residence, the amount of fieldwork was similar in each of the 12 communities, rather than being proportional to the community-specific prevalence of HIV. The selection of individuals rather than matched pairs was logistically simpler. The confounding effects of age, community of residence and other factors were controlled for in the analysis, rather than in the design. The controls also provided a survey of sexual behaviour patterns in a representative sample of this population.

The findings of this study, however, are subject to the same limitations as all case–control studies. First, the results may be biased if the controls do not represent those who would have been selected as cases, had they satisfied the case definition. In our study, cases and controls were drawn from the same source population and the participation rates were broadly similar in cases and controls. Bias may also result if cases and controls differ in their abilities to recall or report. Information on past and current sexual behaviour is particularly prone to recall and reporting bias. In this population, most people are not aware of their HIV status, and most participants of this study chose not to be told their HIV status. Furthermore, interviewers were not aware of the participant's HIV status. Recall and reporting bias are therefore likely to be non-differential and so OR will tend to be underestimated. A further problem arising from case–control studies is the difficulty in determining temporal sequences of events. Our study is based on prevalent HIV cases. Therefore, information pertaining to the past year may not be relevant to an individual's HIV status, or may even represent the effects of HIV infection rather than its causes. This problem may be addressed by using incident HIV cases. We are currently conducting a case–control study based on incident cases of HIV infection in these communities.

Our study identified particular groups of men and women with a high HIV prevalence. First, the prevalence of HIV was higher in men and women who were divorced or widowed than in those currently married. In those aged 30–54 years, marriage was associated with the lowest HIV prevalence, the highest prevalence being in those never married. In those under 30 years, however, married men had a higher HIV prevalence than never-married men. Our findings are consistent with those from rural Uganda [11], except that we did not observe an increased prevalence of HIV infection among young married women. At younger ages, marriage may be a marker of recent sexual activity. However, being divorced, widowed or never married may reflect greater exposure to HIV-infected partners, as suggested by the higher number of casual sexual partners reported in these groups: in male controls (female controls), the proportions reporting three or more (one or more) casual partners in the past year were 33% (25%) in the widowed or divorced, 26% (28%) in the never married, and 11% (7%) in the currently married. Alternatively, it may be that HIV infection was the cause of divorce or widowhood.

An important finding from this study is that some married men and women may have been infected with HIV by their spouse. Women married to men in occupations other than farming had a significantly higher prevalence, as did men married to younger women. Our data do not indicate whether the spouse was the source of HIV infection in the index case. However, these associations persisted even after adjustment for the sexual behaviour of the index case. Furthermore, it is notable that many of the cases whose own sexual behaviour would be categorized as 'low risk' had spouses falling into 'high risk' categories: in women, 56% of cases reporting one or fewer lifetime partners, 33% of cases reporting two or more lifetime partners, and 14% of controls were married to men in manual work, office work or business; in men, 58% of cases reporting four or fewer lifetime partners, 39% of cases reporting five or more lifetime partners, and 37% of controls were married to women aged 24 years or younger. Thus it is possible that a husband in an occupation other than farming, with a risky lifestyle, is the source of HIV infection in some women. This is con-

sistent with the belief that many African women are at increased risk of HIV infection through their spouse [1,17–20]. Likewise, in some men, it is possible that the source of HIV infection may be their younger wife. This is consistent with the high HIV prevalence among young women in this and other studies [4,7–11,14]. It is also consistent with the strong association between HIV and reported sexual partners among the women in this and other studies [7,8,18], and with evidence suggesting that some African women are driven to exchange sex for means of subsistence [21].

In these rural communities, most men and women have primary education or less, most men and women are farmers, and women tend not to travel, either to Mwanza or other large towns (Table 3). The groups who appear to be at particularly high risk of HIV infection are those not currently employed in farming, and the small group of women who travel or have secondary or higher education. These high-risk groups have been identified in other studies [5,7,8,11] and may be markers of more modern lifestyles or higher disposable incomes, which may be associated with risky behaviour. In contrast to other studies [11,12], we found the highest HIV prevalence in the small group of Moslems; in women, this excess risk was statistically significant. The Moslems in this study comprised only a few individuals and may not be representative of the wider population of Moslems. It is also worth noting that the higher prevalence in Moslems could not be attributed to polygamy, since in our study polygamy was not strongly associated with being a Moslem or HIV prevalence. A possible explanation for the increased prevalence, which is consistent with the findings of another study [8], is that Moslems were more likely to have lived in roadside settlements and large towns in the past 5 years, where the risk of HIV infection is greatest [7,14].

Blood transfusions were associated with a twofold increased in risk among women, although only a small population-attributable fraction (4%). The population-attributable fraction associated with at least one injection in the past year was 24% in men and 21% in women. These are likely to overestimate the true population-attributable fraction, since injections in the past year may be an effect of HIV-related illness rather than a route of HIV transmission. No data were collected on history of illnesses, so it was not possible to evaluate this association further. Skin incisions and tattoos were not significantly associated with HIV in men or women and are therefore unlikely to be a major route of HIV transmission.

Particular patterns of reported sexual behaviour were associated with an increased prevalence of HIV infection. As in other studies [4,5,7,8,12,18,19], there was an increased prevalence in those reporting higher numbers of lifetime partners. This association was particularly strong among women, in whom there was a sevenfold increased prevalence associated with 10 or more lifetime partners. The population-attributable fraction for women, associated with 10 or more lifetime partners (9.8%), is consistent with the finding of 9.4% among family planning clinic attenders in Nairobi [18]. In our study, the population-attributable fraction for reporting two or more lifetime sex partners was 66% in men and 50% in women; these figures are similar to those found in rural Uganda (53% in men and 69% in women) [8]. Given the limitations of recall and reporting, and the fact that some partnerships may have occurred long before the HIV epidemic, it is perhaps surprising that the association is so strong. Lifetime sexual history may be acting as a proxy for sexual behaviour during the past few years. In women, reported sexual behaviour in the past year was associated with an increased HIV prevalence, except when it was adjusted for lifetime partners. In men, however, reported sexual behaviour in the past year was not associated with HIV prevalence. This applied irrespective of whether we analysed total number of partners, or number of partners of any type, including short-term partners, commercial sex workers and other casual partners. There was even a significant negative trend of HIV prevalence with increasing partners and increasing casual partners in the past year. An obvious explanation for these findings is that sexual behaviour in men changed as a result of their HIV infection. Since awareness of HIV status is low in this population, changes in behaviour are more likely to be due to HIV-related illness or to the loss of a regular partner due to HIV/AIDS.

There was no increase in HIV prevalence associated with reported sex during travel away from home, or at traditional events. Few men reported sex with commercial sex workers in the past year, although there is a substantial amount of casual sex, and we cannot exclude the possibility that this sometimes involves an element of exchange. However, outside the towns and truck-stops, there are no clearly defined groups of commercial sex workers who could form the basis for targeted intervention programmes. Few women reported the practice of 'dry sex', so it was not possible to estimate its association with HIV infection, as suggested by some other studies [22]. Bruising during sex was reported by 25% of male controls and 10% of female controls, but was not associated with HIV prevalence, in contrast to a study in Jamaica [23]. Interpretation of the association between HIV and history of genital ulcer or discharge in the past year is subject to the same limitations as in other case–control or cross-sectional studies [6–8,11,12]: first, it is possible that this association reflects the confounding effect of some aspect of sexual behaviour for which we have not fully adjusted; second, it is difficult to determine the temporal sequence of these factors.

Many studies have reported a lower risk of HIV and other STD in circumcised men, although some other studies have given conflicting results [24]. Our findings clearly demonstrate the importance of adjusting for confounders. On unadjusted analysis, there was no association between HIV prevalence and circumcision. However, circumcision was strongly associated with several other risk factors, including age, community, religion, marital status and current occupation. Most notably, 64% of non-farmers and only 26% of farmers were circumcised, and 78% of Moslems and only 29% of non-Moslems were circumcised. After adjusting for confounders, circumcision had a modest but non-significant protective effect. The substantial change in the OR after adjustment suggests that there may be residual confounding, for which we have been unable to adjust [24].

Our data suggest that circumcision at age 15 years or older was protective, whereas circumcision at a younger age was associated with an increased HIV prevalence. We cannot explain the greater apparent protective effect among men circumcised at older ages, which was surprising given that some of these men may have been circumcised after onset of exposure to HIV or other STD. Our results contrast with findings in rural Uganda [8] where Moslems, who are usually circumcised in childhood, had a lower prevalence of HIV infection. Although our study population included a suitable mix of circumcised and non-circumcised men, facilitating analysis of the effects of circumcision, the circumcised men formed a heterogeneous group. Men may have been circumcised for different reasons, which may have been differentially associated with their HIV risk.

The main conclusions of this study are, first, that most HIV infections in these rural communities occur through sexual transmission, although some may be due to non-sterile injections. The population-attributable fraction associated with blood transfusions or other routes of infection is small. Interventions to change sexual behaviour patterns are clearly a major priority. Some men and women are at high risk of HIV infection through large numbers of sex partners. Intervention strategies, therefore, should aim to reduce partner change and to promote condom use. However, some men and women with a low-risk profile, most notably those with few sex partners, may be at risk through their spouse or regular partner. This suggests that interventions should extend beyond the high-risk groups. The practice of dry sex and bruising during sex do not seem to play an important role in HIV transmission, whereas the role of male circumcision is unclear. Although a substantial proportion of the population are engaged in high-risk behaviour, commercial sex, as observed in urban surroundings, seems to play a negligible role in HIV transmission. Our results confirm marked heterogeneity in the risk of HIV infection in these rural communities, indicating the scope for risk-reduction strategies.

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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	1.01–1.37
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 of 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 serconversions 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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HIV and male circumcision—a systematic review with assessment of the quality of studies

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This Cochrane systematic review assesses the evidence for an interventional effect of male circumcision in preventing acquisition of HIV-1 and HIV-2 by men through heterosexual intercourse. The review includes a comprehensive assessment of the quality of all 37 included observational studies. Studies in high-risk populations consisted of four cohort studies, 12 cross-sectional studies, and three case-control studies; general population studies consisted of one cohort study, 16 cross-sectional studies, and one case-control study. There is evidence of methodological heterogeneity between studies, and statistical heterogeneity was highly significant for both general population cross-sectional studies (χ^2=132·34; degrees of freedom [df]=15; p<0·00001) and high-risk cross-sectional studies (χ^2=29·70; df=10; p=0·001). Study quality was very variable and no studies measured the same set of potential confounding variables. Therefore, conducting a meta-analysis was inappropriate. Detailed quality assessment of observational studies can provide a useful visual aid to interpreting findings. Although most studies show an association between male circumcision and prevention of HIV, these results may be limited by confounding, which is unlikely to be adjusted for.

According to the latest UNAIDS estimates, 42 million people were living with HIV/AIDS in 2002.[1] Half of these people were women and 3·2 million were children younger than 15 years old. Almost 30 million people living in Africa are affected by HIV/AIDS and 2·4 million Africans died of AIDS during 2002.[1] Sub-Saharan Africa is by far the worst affected region, and the national adult prevalence rates exceed 30% in the southern African countries of Botswana, Lesotho, Swaziland, and Zimbabwe. In South Africa, HIV/AIDS accounts for 38% of years of life lost and is the major contributor to disability-adjusted life years in adults.[2]

Given the enormous mortality and morbidity associated with HIV/AIDS, it seems reasonable to fully explore potential prevention measures. For over a decade many observational studies have suggested a protective effect of male circumcision (figure 1) on HIV acquisition in men. These findings are supported by the biological theory that the entry of HIV into host cells is facilitated by CD4 and other HIV coreceptors present on the Langerhans' cells of the foreskin.[3–5] Six reviews[6–11]—including two meta-analyses[9,10]—of these observational studies have reached different conclusions on the association between male circumcision and HIV infection. Search strategies were not clearly described in all the reviews, several focused only on published studies, and confounding was not always adequately assessed. None of the reviews reported on the methodological quality of included studies.

The most rigorous of these reviews is a systematic review and meta-analysis of 27 published studies on HIV-1 infection in sub-Saharan Africa by Weiss and colleagues,[10] published in 2000. Adjusted analyses produced odds ratios (ORs) indicating a benefit of circumcision: OR=0·42 (95% CI 0·34–0·54) for all studies combined (n=15); OR=0·55 (95% CI 0·42–0·72) for population-based cross-sectional studies (n=5); and OR=0·24 (95% CI 0·18–0·31) for cross-sectional studies of high-risk groups (n=4). Because ORs were less than 1, the authors concluded that there was compelling evidence of a substantial protective effect of male circumcision against HIV infection in sub-Saharan Africa, while warning that residual confounding may exist in some studies because of unknown or unmeasured behavioural or biological factors. In a review of 48 published observational studies (including studies of homosexual men), Bailey and co-workers[11] described confounding variables potentially present in these studies in general, but did not report on the quality of each included study.

We report updated results from a Cochrane systematic review in which we assessed the likelihood that male circumcision reduces acquisition of HIV-1 and HIV-2 in heterosexual men, first published in 2003.[12] We evaluate the methodological quality of each included study and quantify the level of heterogeneity between studies.

Methods

Search strategy and selection criteria

We planned to include randomised or quasirandomised controlled trials. Should data be insufficient—ie, no randomised controlled trials identified—data from

Allan Jeffries

Figure 1: **A face painted with white clay and a traditional blanket identify this Xhosa youth as an initiate**
During a period known as "ulwaluko" (male initiation), he will be ritually circumcised and instructed in the ways of manhood to be received and perceived as a man. Permission of the individual was obtained for this photograph.

observational studies (cohort, case-control, and cross-sectional studies) would be considered for inclusion in this review. Studies done in general or specific populations and in hospitals or clinics were included. Studies done in any country and published in any language were included. Studies with historic controls and ecological studies were excluded, because these studies provide less reliable data for assessing association.

We searched online for published and unpublished studies in the Cochrane controlled trials register, Medline, Embase, and Gateway/Aidsline in 2002, and again in November 2004. We also searched databases of conference abstracts, scanned reference lists of articles, and contacted authors of included studies and researchers working in the field to source unpublished studies. The full search strategy is described elsewhere.[12] Reviewers independently screened each record for eligibility by examining titles, abstracts, and keywords. Two reviewers independently applied the inclusion criteria using a standard form, and differences were resolved by discussions with a third reviewer.

Data extraction and outcome measures

Two reviewers independently extracted data on the type of study and the participants in the study. Only studies that included participants defined as heterosexual males 12 years of age or older were included. Studies of discordant couples were excluded. We also recorded whether the intervention—circumcision—was a medical intervention or done for cultural or religious practices, and whether circumcision status was determined by self-report, partner-report, or direct observation. The primary outcome was HIV-1 or HIV-2 infection (incidence or prevalence) in men, based on laboratory results. The specific tests used to ascertain and confirm HIV status were recorded, as well as the reporting of ten possible confounding factors (panel). We reported any medical adverse events associated with circumcision if recorded in the studies. Reviewers were not blinded to the names of the authors, institutions, journal of publication, or results of the studies.

A number of the included studies are described in more than one publication. In some cases, additional analyses conducted after completion of a study were reported. Where methods of study design were described in additional publications, we used all reports to inform our data extraction. Where additional analyses were conducted, we chose to include the analysis that provided the most information and avoided duplication of results. The full description is available in the Cochrane review.[12]

Data analysis and statistical methods

We used REVMAN software to analyse our data. For each study, we expressed findings as crude and adjusted ORs with their 95% CIs. An OR below 1 indicated a protective effect of circumcision. Statistical significance was indicated by p values less than 0·05. The χ^2 test for heterogeneity was used to provide an indication of between-study heterogeneity (statistical significance was taken as $p<0·1$). In addition, the degree of heterogeneity observed in the results was quantified using the I^2 statistic,[13] which can be interpreted as the percentage of variation observed between the studies caused by between-study differences rather than chance. Studies are presented stratified by study design, further stratified by general population or high-risk groups. High-risk groups included participants who are considered at greater risk of contracting HIV due to the nature of their lifestyle and activities—eg, truck drivers, men who have sex with sex workers, patients attending sexually transmitted infection (STI) clinics.

Methodological quality of included studies

We developed a standardised quality assessment form for observational studies specifically for the review. The form included three separate sections for cohort studies, cross-sectional studies, and case-control studies. We appraised the quality of each study using a "star system".[14] This system included appraisal of external and internal validity and biases relevant to observational studies in general, and specific to circumcision and HIV. Two reviewers independently evaluated study quality and differences were resolved by discussions with a third reviewer.

Results

We identified three randomised controlled trials currently underway in Africa. We included 37 observational studies: 18 conducted in the general population and 19 in high-risk populations. Two new studies not included in the original review and one updated study were identified. Meta-analysis was not done because many of the studies had a high likelihood of bias and there was substantial heterogeneity, suggesting that any overall summary statistic could be misleading. Synthesis focused on describing the direction and consistency of effect, assessing the likelihood of bias, and investigating factors that may explain differences between the results of studies. No

***Panel*: Potential confounding factors**

Age
Location of study (eg, rural, urban)
Religion
Education, occupation, and socioeconomic status
Sexual behaviour (eg, measured by age at first intercourse, number of sexual partners, contact with sex workers)
Any STIs
Condom use
Migration status
Travel to different countries
Other possible exposures (eg, injections, blood transfusions, homosexual intercourse)

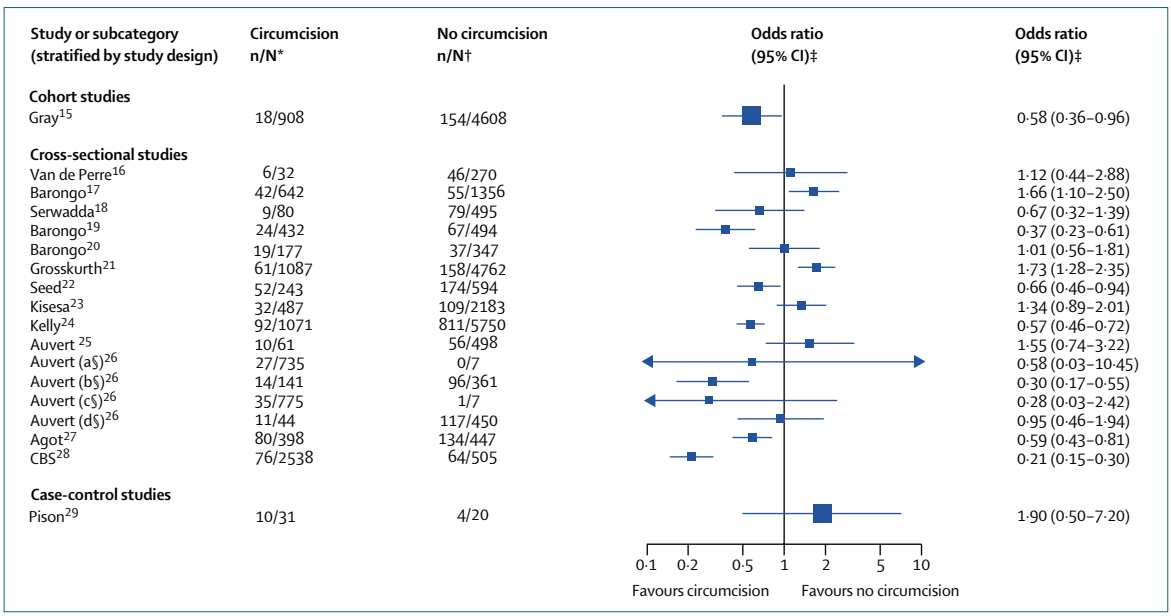

Figure 2: **Crude results of general population studies assessing HIV and circumcision status**
The point estimate (odds ratio, OR) for each study is represented by a square. The 95% CI for each study is represented by a horizontal line intersecting the square. The size of the square represents the relative precision of the study estimates within each study design strata. The data are displayed on a logarithmic scale. *n/N represents the number of HIV-positive participants (n) in the circumcised group over the total number of participants (N) in the circumcised group. †n/N represents the number of HIV-positive participants (n) in the uncircumcised group over the total number of participants (N) in the uncircumcised group. ‡Odds ratio and 95% CI. ORs greater than 1 indicate increased risk of HIV infection with circumcision and ORs less than 1 indicate decreased risk of HIV infection with circumcision. §a, b, c, and d represent different studies discussed by Auvert et al.[26]

studies reported on the medical complications of circumcision. In most studies, exposure to circumcision had reportedly taken place during childhood or adolescence, before the studies commenced.

General population study results

We identified one cohort study, 16 cross-sectional studies, and one case-control study conducted in general populations. The crude results are shown in figure 2.

The single cohort study[15] (n=5516) showed a significant difference in HIV transmission rates between circumcised and uncircumcised men (OR=0·58; 95% CI 0·36–0·96). Adjustment for potential confounders did not alter this result.

The 16 cross-sectional studies had inconsistent findings.[16–28] Ten studies indicated circumcision was beneficial whereas six indicated it was harmful, with odds ratios varying between 0·21 and 1·73. Eight studies had statistically significant results, six indicating a benefit and two indicative of harm. The test for heterogeneity was highly significant (χ^2=132·34; df=15; p<0·00001). 89% of the variability observed between the studies was attributable to between-study differences and not random variation (I^2=88·71%). Ten studies reported adjusted ORs, with nine of these studies showing a benefit for circumcision, ranging from OR=0·26 to 0·80. Five of these studies had significant results and three insignificant results. The study that indicated a harmful effect of circumcision reported an adjusted OR of 1·25, but did not report CIs. The studies all adjusted for different sets of potential confounders. Use of adjusted results accounted for only 3% of the unexplained variability in results, 86% of the variability remaining inexplicable. The quality of each study is shown in table 1.

Only one case-control study in a general population setting was identified.[29] This study (n=51) found no significant difference in HIV transmission rates between circumcised and uncircumcised men (OR=1·90; 95% CI 0·50–7·20).

High-risk group study results

We identified four cohort studies, 12 cross-sectional studies, and three case-control studies conducted in high-risk groups (figure 3). One cross-sectional study presented only an adjusted estimate.[35]

Results from the four cohort studies[42–45] all indicated benefit from circumcision and three of them had statistically significant results. Point estimates from crude ORs varied from 0·10 to 0·39. The χ^2 test for between-study heterogeneity was marginal (χ^2=6·17; df=3; p=0·10) and 51% of the variability in results was not explicable by chance (I^2=51·4%).

Crude results from 11 cross-sectional studies were indicative of a benefit from the intervention, eight being statistically significant.[30–34,36–41] Estimates of effect varied from ORs of 0·10 to 0·66. Between-study heterogeneity was significant (χ^2=29·70; df=10; p=0·001). 66% of the

Study	External validity		Internal validity																OR (95% CI)	
			Performance		Detection			Attrition	Selection bias/control of confounding											
	Representative*	Participation rate†	Direct observation	Blinded assessors	1st HIV test	2nd HIV test	Blinded assessors	Completeness‡	Age	Location	Religion	SES/education	Marital status	Sexual behaviour	Any STI	Condom use	Travel/ migration	Other exposure	Crude	Adjusted
General population groups																				
Agot[27]	✓	..	✓	✓	✓	✓	✓	..	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	0·59 (0·34–0·81)	0·48 (0·33–0·67)
Auvert[25]	✓	✓	..	..	✓	..	✓	..	..	✓	..	..	..	..	..	..	..	..	1·55 (0·74–3·22)	
Auvert (a§)[26]	✓	✓	✓	✓	✓	✓	✓	..	..	✓	..	..	..	..		..	..	..	0·58 (0·03–10·46)	
Auvert (b§)[26]	✓	..	✓	✓	✓	✓	✓	..	✓	✓	..	..	✓	✓	✓	✓	✓	..	0·30 (0·17–0·55)	0·26 (0·12–0·56)
Auvert (c§)[26]	✓	..	✓	✓	✓	✓	✓	..	..	✓	..	..	..	..	..	..	..	..	0·28 (0·03–2·42)	
Auvert (d§)[26]	✓	..	✓	✓	✓	✓	✓	..	..	✓	..	..	..	..	..	..	..	..	0·95 (0·46–1·94)	
Barongo[17]	✓	✓	..	..	✓	✓	✓	..	✓	✓	..	..	✓	✓	✓	..	✓	✓	1·6 (1·10–2·50)	0·8 (0·5–1·3)
Barongo[19]	✓	..	..	..	✓	✓	✓	✓	✓	✓	..	✓	✓	..	✓	..	✓	✓	0·37 (0·23–0·61)	0·40 (0·23–0·71)
Barongo[20]	✓	..	..	..	✓	✓	✓	✓	..	✓	✓	✓	..	✓	✓	✓	..	..	0·21 (0·15–0·30)	
Grosskurth[21]	✓	✓	..	✓	✓	✓	✓	✓	✓	✓	..	✓	✓	..	✓	..	✓	✓	1·73 (1·28–2·35)	1·25 (Not reported)
Kelly[24]	✓	..	..	..	✓	✓	✓	✓	✓	✓	..	✓	✓	✓	✓	✓	..	..	0·57 (0·46–0·72)	0·44 (0·35–0·56)
Kisesa[23]	✓	..	..	..	✓	✓	..	✓	..	✓	✓	✓	..	✓	✓	✓	..	..	1·34 (0·89–2·01)	0·66 (0·41–1·08)
Seed[22]	..	..	✓	✓	✓	✓	✓	✓	✓	✓	..	..	..	..	✓	..	..	..	0·66 (0·46–0·94)	0·59 (0·40–0·86)
Serwadda[18]	✓	..	..	..	✓	✓	✓	✓	✓	✓	..	..	..	✓	✓	..	..	..	0·67 (0·32–1·39)	0·4 (0·2–0·9)
Van de Perre[16]	✓	✓	..	..	✓	✓	✓	✓	..	✓	..	..	..	..	..	..	..	..	1·12 (0·44–2·28)	
High-risk groups																				
Bwayo[30]	✓	..	..	..	✓	✓	✓	✓	✓	..	..	✓	✓	✓	✓	✓	..	..	0·24 (0·17–0·34)	0·20 (0·12–0·36)
Diallo[31]	✓	..	✓	✓	✓	✓	✓	✓	..	..	..	✓	..	✓	✓	..	✓	..	0·30 (0·19–0·48)	
Gilks[32]	✓	✓	..	..	✓	✓	✓	✓	..	..	..	..	..	..	..	..	..	..	0·17 (0·09–0·35)	
Greenblatt[33]	✓	..	✓	✓	✓	✓	✓	✓	✓	✓	..	..	..	✓	✓	..	..	✓	0·30 (0·11–0·82)	
Lankoande[34]	✓	✓	✓	✓	✓	✓	✓	✓	..	..	..	..	..	..	..	..	..	..	0·66 (0·23–1·93)	
Mbugua[35]	✓	..	..	..	✓	✓	✓	✓	✓	..	..	✓	..	✓	..	..	✓	..	–	0·27 (0·11–0·65)
Mehendale[36]	✓	..	✓	✓	✓	✓	✓	✓	✓	✓	..	✓	✓	✓	✓	✓	..	✓	0·61 (0·43–0·87)	0·59 (0·41–0·84)
Nasio[37]	..	✓	✓	✓	..	..	✓	✓	..	..	..	..	..	..	✓	..	..	..	0·21 (0·15–0·31)	0·22 (Not reported)
Pepin[38]	..	..	✓	✓	✓	✓	✓	✓	..	..	..	..	..	..	..	..	..	..	0·45 (0·15–1·33)	
Simonson[39]	✓	..	✓	✓	✓	✓	✓	..	..	..	..	..	..	✓	✓	..	✓	..	0·36 (0·18–0·72)	
Tyndal[40]	✓	✓	✓	✓	✓	✓	✓	✓	✓	..	..	..	✓	✓	✓	✓	..	✓	0·22 (0·15–0·31)	0·21 (0·14–0·30)
Vaz[41]	✓	..	..	..	✓	✓	✓	✓	..	..	..	..	..	..	..	..	..	..	0·10 (0·01–1·81)	

SES=socioeconomic status; STI=sexually transmitted infection; ✓indicates the measure was adequately addressed in the study; *studies received a ✓ if the sample included all eligible HIV-negative men over a defined period of time, or in a defined catchment area, or a random or systematic sample of those men; †studies received a ✓ if the percentage participation was 80% or more; ‡studies received a ✓ if the percentage participants in the final analysis was 80% or more, or if a full description of those lost-to-follow-up was not suggestive of bias. For selection bias/control of confounding a ✓ indicates that the group variable was either balanced between groups (10% or less difference) or adjusted for in analysis. §a, b, c, and d represent different studies discussed by Auvert et al.[26]

Table 1: **Quality assessment of cross-sectional studies**

variability in results was not explicable by chance (I^2=66·4%). Five of the cross-sectional studies report adjusted ORs ranging from 0·20 to 0·59, four of these studies were significant and one did not provide data to calculate CIs, although it was reported as a significant OR.[37] None of these studies adjusted for the same set of potential confounders. The quality of each study is shown in table 1.

Three case-control studies met inclusion criteria and all indicated a protective effect of circumcision on HIV status, two being statistically significant.[46–48] ORs varied from 0·37 to 0·88. The test for between-study heterogeneity was marginal (χ^2=4·36; df=2; p=0·11). 54% of the variation in results could not be explained by chance (I^2=54·1%). One study reported an adjusted OR of 0·50 (95% CI 0·30–0·77), adjusting for location, socioeconomic status, marital status, sexual behaviour, any STI, and condom use.[47]

Subgroup analysis

Our decision to stratify results by risk group and study design was supported by the results of the studies. Studies in high-risk groups were significantly more in favour of circumcision than those done in general population studies (p=0·00006 by meta-regression of adjusted results), and differences were observed between study designs for the high-risk studies (p=0·044 for cross-sectional studies compared with case-control studies; p=0·029 for cohort studies compared with case-control studies). Insufficient numbers of cohort and case-control studies were included to make the same comparison among general population studies.

We were able to do a subgroup analysis on mode of establishing circumcision status: self-report versus direct observation. Because of the small number of studies in some strata, it was only possible to assess cross-sectional studies within the general population group (figure 4). All six cross-sectional studies using direct observation indicated a benefit of circumcision (OR 0·28–0·95), with three of the studies indicating a significant benefit. The ten studies based on self-report described a mixture of benefit (four studies) and harm (six studies) with OR ranging from 0·21 to 1·88. Between-study heterogeneity was substantial in the subgroup of self-reported studies

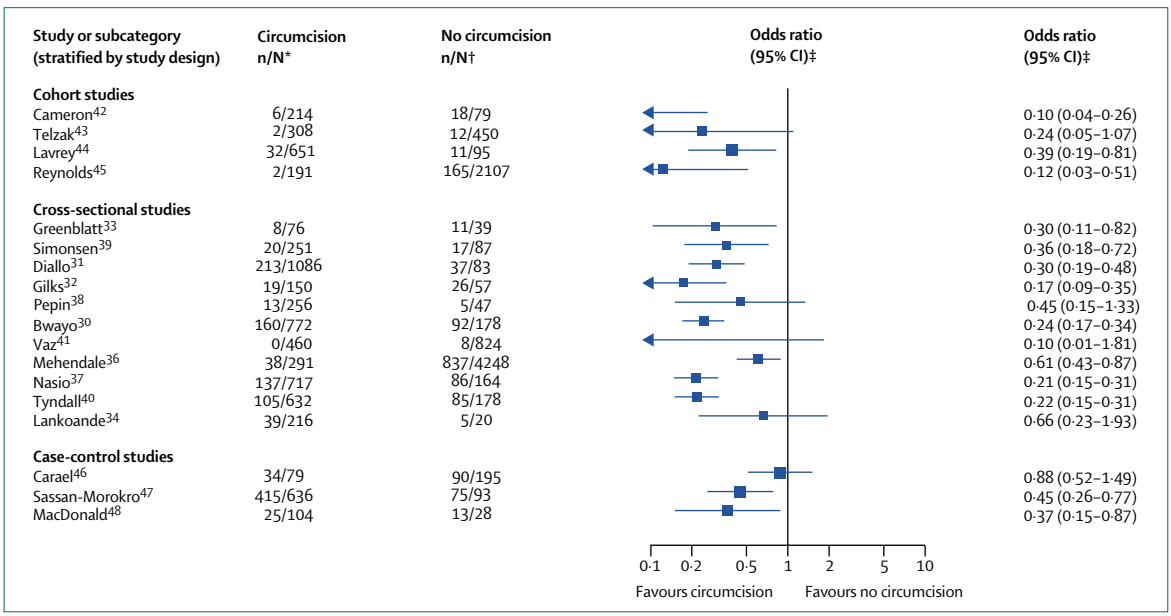

Figure 3: **Crude results of high-risk group studies assessing HIV and circumcision status**
The point estimate (odds ratio, OR) for each study is represented by a square. The 95% CI for each study is represented by a horizontal line intersecting the square. The size of the square represents the relative precision of the study estimates within each study design strata. The data are displayed on a logarithmic scale. *n/N represents the number of HIV-positive participants (n) in the circumcised group over the total number of participants (N) in the circumcised group. †n/N represents the number of HIV-positive participants (n) in the uncircumcised group over the total number of participants (N) in the uncircumcised group. ‡Odds ratio and 95% CI. ORs greater than 1 indicate increased risk of HIV infection with circumcision and ORs less than 1 indicate decreased risk of HIV infection with circumcision.

(χ^2=135·23; df=9; p<0·00001; I^2=93%), but marginal in the direct observation subgroup (χ^2=7·20; df=5; p=0·21; I^2=31%). The difference between the groups did not reach statistical significance (p=0·27). Results from studies using direct observation were still heterogeneous, 31% of the observed variability not being explicable by chance.

We were not able to conduct subgroup analysis on HIV-1 versus HIV-2 status, because many studies did not clearly report on the type of HIV, and those studies that measured both often did not differentiate between the two types in analysis. 21 of the studies assessed HIV-1 status only, one study only included HIV-2, six studies included both HIV-1 and HIV-2, and six studies were unclear whether HIV-1 or HIV-2 was measured.

We were not able to conduct subgroup analysis on background prevalence of HIV in the sampled populations because this information was unavailable for almost all studies.

Quality of included studies

The overall study quality was highly variable (tables 1–3). Performance bias (misclassification of exposure) may be present in all studies where circumcision status was obtained by self-report rather than direct observation.

Study	External validity	Internal validity																			OR (95% CI)	
		Performance			Detection			Attrition	Selection bias/control of confounding													
	Representative	Participation rate*	Direct observation	Blinded assessors	1st HIV test	2nd HIV test	Cases=control†	Completeness‡	Case selection	Control selection	Age	Location	Religion	SES/education	Marital status	Sexual behaviour	Any STI	Condom use	Travel/migration	Other exposure	Crude	Adjusted
General population groups																						
Pison[29]	✓	..	..	✓	✓	✓	✓	✓	✓	✓	✓	✓	..	..	✓	✓	✓	✓	✓	✓	1·90 (0·50–7·20)	
High-risk groups																						
Carael[46]	..	..	..	✓	✓	✓	✓	✓	..	..	✓	..	..	..	..	✓	..	..	✓	..	0·88 (0·52–1·49)	
MacDonald[48]	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	..	..	✓	..	✓	✓	..	..	..	0·37 (0·15–0·87)	
Sassan-Morokro[47]	..	..	..	✓	..	..	..	✓	✓	✓	..	✓	..	✓	✓	✓	✓	✓	..	✓	0·45 (0·26–0·77)	0·50 (0·30–0·77)

SES=socioeconomic status; STI=sexually transmitted infection; ✓indicates the measure was adequately addressed in the study; *studies received a ✓ if the percentage participation was 80% or more; †studies received a ✓ if the same method of ascertainment was used for cases and controls; ‡studies received a ✓ if the percentage participants in the final analysis was 80% or more, or if a full description of those lost-to-follow-up was not suggestive of bias. For selection bias/control of confounding a ✓ indicates that the group variable was either balanced between groups (10% or less difference) or adjusted for in analysis.

Table 2: **Quality assessment of case-control studies**

17 studies assessed circumcision status by self-report and 20 by direct observation. Detection bias (misclassification of outcome) was unlikely, because nearly all studies (n=35) used blinded methods for assessing and confirming HIV status. All five cohort studies included in the review were classified as susceptible to attrition bias as loss-to-follow-up was either greater than 20%,[44] unequal between circumcised and uncircumcised groups,[42] not reported, or unclear.[15,43,45]

Selection bias was problematic in all studies. Circumcised and uncircumcised groups (in cohort and cross-sectional studies) and HIV-positive and HIV-negative groups (in case-control studies) were seldom balanced (less than 10% difference between circumcised and uncircumcised groups) for all or most of the ten risk factors that we identified as potential confounders before the quality assessment. Statistical adjustments for measured confounding factors were made in only 20 of the 37 included studies. The adjusted confounders differed across studies in number and type.

Discussion

There are currently no completed randomised controlled trials assessing the effectiveness of male circumcision in preventing HIV acquisition in heterosexual men. However, three large trials have commenced in Kenya (n=2776), Uganda (n=5000), and South Africa (n=3500), and are scheduled for completion in 2006–2007. 37 observational studies met the review inclusion criteria: 18 conducted in the general population and 19 in high-risk groups.

Methodological issues

The strengths of this review are its comprehensive coverage, our assessment of the biases often found in traditional narrative reviews,[49] and our extensive assessment of the quality of existing studies. Firstly, to reduce publication and language bias, we conducted an extensive search to source all studies, regardless of publication status or language. Secondly, we did not limit the review to studies conducted in a particular geographic region and included both HIV-1 and HIV-2 infection. We therefore included 37 studies, making this the largest systematic review of male circumcision and heterosexual transmission of HIV to date. Thirdly, we undertook an appraisal of the quality of all included studies using a quality assessment tool specifically developed for this review. This tool allowed for an intense interrogation of the quality of each study and let us make a more informed judgment regarding the appropriateness of pooling the results in a meta-analysis.

Observational studies, unlike randomised controlled trials, can only adjust for known confounders and only those that are measured without error.[50] In assessing the quality of the observational studies we identified ten potentially important confounders (panel). Many studies either did not measure or report these variables. Where confounders were reported, they were often not balanced between groups or not adjusted for. Religion commonly fell into this category. Among studies that did report confounders, choice of potential confounders was highly variable across studies. The effect of unknown

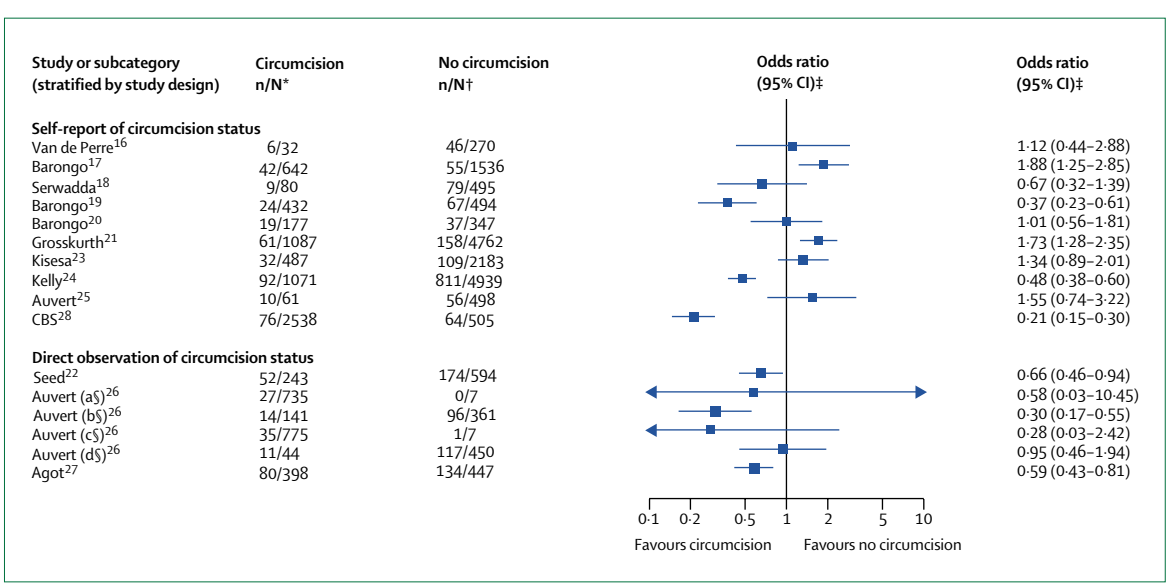

Figure 4: **Crude results of cross-sectional general population studies assessing HIV and circumcision status: self-report of circumcision *vs* direct observation**
The point estimate (odds ratio, OR) for each study is represented by a square. The 95% CI for each study is represented by a horizontal line intersecting the square. The size of the square represents the relative precision of the study estimates within each study design strata. The data are displayed on a logarithmic scale. *n/N represents the number of HIV-positive participants (n) in the circumcised group over the total number of participants (N) in the circumcised group. †n/N represents the number of HIV-positive participants (n) in the uncircumcised group over the total number of participants (N) in the uncircumcised group. ‡Odds ratio and 95% CI. ORs greater than 1 indicate increased risk of HIV infection with circumcision and ORs less than 1 indicate decreased risk of HIV infection with circumcision. §a, b, c, and d represent different studies discussed by Auvert et al.[26]

Study	External validity		Internal validity																OR (95% CI)	
			Performance	Detection			Attrition	Selection bias/control of confounding												
	Representative*	Participation rate†	Direct observation	1st HIV test	2nd HIV test	Blinded assessors	Equal follow-up‡	Completeness§	HIV-negative at commencement	Age	Location	Religion	SES/ Education	Marital status	Sexual behaviour	Any STI	Condom use	Travel/ migration	Other exposure	Crude	Adjusted
General population groups																				
Gray[15]	✓	✓	..	✓	✓	✓	..	..	✓	✓	✓	..	✓	✓	✓	✓	✓	..	..	0·58 (0·36–0·96)	0·53 (0·33–0·87)
High-risk groups																				
Cameron[42]	✓	✓	✓	✓	✓	✓	✓	..	✓	..	..	..	..	..	✓	✓	..	..	..	0·10 (0·04–0·26)	0·12 (0·04–0·33)
Lavrey[44]	✓	✓	✓	✓	✓	✓	✓	..	✓	..	..	..	✓	✓	✓	✓	..	..	..	0·39 (0·19–0·81)	
Reynolds[45]	✓	..	✓	✓	✓	✓	..	..	✓	✓	✓	..	✓	✓	✓	✓	✓	..	✓	0·12 (0·03–0·51)	0·15 (0·04–0·62)
Telzak[43]	..	..	✓	✓	✓	✓	..	..	✓	..	✓	..	..	..	✓	✓	..	..	..	0·24 (0·05–1·07)	0·29 (0·06–1·25)

SES=socioeconomic status; STI=sexually transmitted infection; ✓indicates the measure was adequately addressed in the study; *studies received a ✓ if the sample included all eligible HIV-negative men over a defined period of time, or in a defined catchment area, or a random or systematic sample of those men; †studies received a ✓ if the percentage participation was 80% or more; ‡studies received a ✓ if both groups were followed-up for the same amount of time or within 10% of each other; §studies received a ✓ if the percentage participants in the final analysis was 80% or more or if a full description of those lost-to-follow-up was not suggestive of bias. For selection bias/control of confounding a ✓ indicates that the group variable was either balanced between groups (10% or less difference) or adjusted for in analysis.

Table 3: **Quality assessment of cohort studies**

confounders may well be operating in either direction within and across all of the included studies. Furthermore, misclassification of confounders can greatly hinder the effectiveness of any statistical adjustment procedure.[51]

We observed differences in results according to study design, confirming that study design is an important consideration in the interpretation of results. Also, we noted that the method of ascertaining circumcision status had an influence on study results, with studies using direct observation consistently reporting a protective effect of circumcision. How much the results are influenced by other aspects of study quality is unclear.

Although use of adjusted results tended to show stronger evidence of an association than the crude results in general population studies, adjustment explained very little of the substantial between-study heterogeneity. Population studies done with direct observation were more in favour of circumcision. Since self-report of circumcision status may be a poor means of assessing exposure,[52] it would seem reasonable to favour the results generated from those studies that used direct observation only. Self-report could affect the results in either direction depending on what the reason for over-reporting or under-reporting in a particular setting is.

When assessing the effects of interventions, it is important to note that observational studies differ in two key ways from randomised controlled trials. Firstly, the intervention (circumcision) did not occur as part of the study, nor was it likely that it occurred directly for reason of possible HIV prevention. Most study participants were likely to be circumcised for cultural or religious reasons. Secondly, the studies were not designed to have comparable circumcised and non-circumcised groups. Since HIV is related to sexual behaviour, which may in turn be partly determined by culture and religion, strong confounding in these studies seems likely. Circumcision itself may be a proxy measure of the knowledge and behaviour learnt during the process of initiation, in which time young men are taught about traditional sexual practices, including monogamy, and penile hygiene (figure 1). Worth noting is that the possible adverse effects of circumcision, such as haemorrhage, infection (including the transmission of HIV), and fistula, were not reported in any of the included studies.[53] No studies measured the acceptance, or otherwise, of circumcision by the sampled communities.

Comparison with other studies

Our review aimed to assess the interventional benefit of male circumcision in reducing HIV acquisition in heterosexual men. The observational studies of high-risk groups included in our review show a strong association between circumcision and reduced rates of HIV acquisition, measured by both crude and adjusted ORs. These results are in accordance with the findings of Weiss and colleagues,[10] who included eight cross-sectional studies in their meta-analysis of the crude results in high-risk groups (OR=0·24; 95% CI 0·20–0·29) and those of a review by Bailey and co-workers.[11] Like Weiss and colleagues, we found a high degree of statistical heterogeneity in population-based cross-sectional studies when only crude results were considered. However, we chose not to conduct a meta-analysis within any of the study categories, based on our findings of the inherent methodological and statistical heterogeneity between studies and the variable quality of all the included studies.

Limitations of the review

Despite our rigorous methods, the review is still subject to a number of limitations. The review may be prone to indexing bias, publication bias, and reporting bias.[49] Our initial search strategy was limited to the term

Search strategy and selection criteria

The search strategy and criteria for selection are described in detail in the Methods section.

"circumcision", which yielded between 143 and 360 abstracts, depending on the database searched. However, when the search included the broader term "risk factors", the yield was over 12 000 abstracts. Appraisal of this many abstracts was not considered feasible. Therefore, it is possible that studies appraising circumcision, but not indexed as such, may have been missed. Although every effort was made to trace unpublished studies, we were not always able to track down authors of abstracts presented at conferences organised during the 1980s and early 1990s.

Reporting bias may have affected our study, as well as other published reviews. Unless we were able to contact researchers to obtain missing data, we relied on the information reported in the article. In many cases reporting was unclear regarding factors relating to study quality, provision of actual numbers, percentages, and details of statistical analyses. Some studies may have included circumcision as a risk factor and, on finding it to be not significant, failed to report on it. In general, we chose to report unclear issues as such, rather than making assumptions. Where necessary, we have been explicit about assumptions that we have had to make. The strength of the review could be greatly improved if it were possible to contact all researchers and obtain summary, or even individual person, data on outcome, exposure, and potential confounders.

Conclusion

The possibility exists that the observed results included in this review could be explained by confounding. Although the positive results of these observational studies suggest that circumcision is an intervention worth evaluating in randomised controlled trials, the current quality of evidence is insufficient to consider implementation of circumcision as a public-health intervention. Therefore, the results of the three randomised controlled trials underway will provide essential evidence about the effects of male circumcision as an intervention to prevent HIV infection. Doing detailed quality assessment of observational studies can aid decision-making about doing a meta-analysis and assist interpretation of results in systematic reviews.

Conflicts of interest

ME has researched circumcision previously in publications in the public domain. No reviewers are part of any of the trial groups investigating the link between circumcision and HIV.

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Randomized, Controlled Intervention Trial of Male Circumcision for Reduction of HIV Infection Risk: The ANRS 1265 Trial

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Author Contributions: BA designed the study with DT, EL, and AP. DT and AP were responsible for operational aspects, including laboratory and field work and in-country administration of the study. BA monitored the study with input from EL and wrote the paper with input from all authors. BA analyzed the data with RS, with inputs from JST. RS conducted the interim analysis.

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Abbreviations: AE, adverse event; IQR, interquartile range; M[number], month [number]; MC, male circumcision; py, person-year; RR, rate ratio; STI, sexually transmitted infection; VCT, voluntary counselling and testing

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ABSTRACT

Background

Observational studies suggest that male circumcision may provide protection against HIV-1 infection. A randomized, controlled intervention trial was conducted in a general population of South Africa to test this hypothesis.

Methods and Findings

A total of 3,274 uncircumcised men, aged 18–24 y, were randomized to a control or an intervention group with follow-up visits at months 3, 12, and 21. Male circumcision was offered to the intervention group immediately after randomization and to the control group at the end of the follow-up. The grouped censored data were analyzed in intention-to-treat, univariate and multivariate, analyses, using piecewise exponential, proportional hazards models. Rate ratios (RR) of HIV incidence were determined with 95% CI. Protection against HIV infection was calculated as $1 - \text{RR}$. The trial was stopped at the interim analysis, and the mean (interquartile range) follow-up was 18.1 mo (13.0–21.0) when the data were analyzed. There were 20 HIV infections (incidence rate = 0.85 per 100 person-years) in the intervention group and 49 (2.1 per 100 person-years) in the control group, corresponding to an RR of 0.40 (95% CI: 0.24%–0.68%; $p < 0.001$). This RR corresponds to a protection of 60% (95% CI: 32%–76%). When controlling for behavioural factors, including sexual behaviour that increased slightly in the intervention group, condom use, and health-seeking behaviour, the protection was of 61% (95% CI: 34%–77%).

Conclusion

Male circumcision provides a degree of protection against acquiring HIV infection, equivalent to what a vaccine of high efficacy would have achieved. Male circumcision may provide an important way of reducing the spread of HIV infection in sub-Saharan Africa. (Preliminary and partial results were presented at the International AIDS Society 2005 Conference, on 26 July 2005, in Rio de Janeiro, Brazil.)

Introduction

Male circumcision (MC) is associated with various cultural factors, including religious sacrifice, rites of passage into adulthood, and the promotion of hygiene. The earliest documentary evidence for circumcision is from Egypt. Tomb artwork from the Sixth Dynasty (2345–2181 B.C.) shows circumcised men, and one relief from this period shows the rite being performed on a standing adult male. Genesis (17:11) places the origin of the rite among the Jews in the age of Abraham, who lived around 2000 B.C.

Presently, MC practices in Africa are varied. Whereas men in Muslim countries are circumcised, as in North Africa or a large part of West Africa, in other societies the prevalence of MC depends on other cultural factors, such as changes that occurred under colonization. In countries such as Cameroon and the Democratic Republic of Congo, which are predominately non-Muslim, most men are circumcised [1–3]. In Kenya, where only a minority of men are Muslims, men in all tribes except the Luo practice MC [4].

The first paper suggesting a protective effect of MC against HIV infection was published in 1986 [5]. Since then, many observational studies have been published, some of which have observed that most men living in East and southern Africa, the regions with the highest prevalence of HIV, are not circumcised [1–3]. A majority of these observational studies are cross-sectional, and a minority are prospective [6–11]. A systematic review and meta-analysis found that in sub-Saharan Africa MC is associated with a significantly reduced risk of HIV infection among men, with an adjusted relative risk of 0.42 (95% CI: 0.34%–0.54%) [12].

All of these studies were based on observational data, and, in the absence of experimental studies, a causal relationship between MC and protection against HIV infection could not be determined [13]. Direct experimental evidence is needed to establish this relationship and, should a protective effect of MC be proven, to convince public health policy makers of the role of MC in reducing the spread of HIV [7,13,14].

The primary objective of this study was to determine the impact of MC on the acquisition of HIV by young men through a randomized, controlled, blindly evaluated intervention trial. The secondary objective was to assess the role of behavioural factors known to be associated with HIV serostatus in explaining the possible impact. This study was conducted in the Gauteng province of South Africa, where HIV prevalence among pregnant women was estimated to be 29.6% in 2003 [15]. According to an earlier study in the research site area, 59% (95% CI: 55%–63%) of uncircumcised men said that they would be circumcised if it reduced their chance of acquiring HIV and STDs [16].

Methods

General Presentation

A randomized, controlled, blindly evaluated intervention trial was carried out in Orange Farm and surrounding areas, a semi-urban region close to the city of Johannesburg. The recruitment of participants took place in the general population from July 2002 to February 2004. Information about the trial was disseminated in the community through meetings during the recruitment period. Precise oral and written information was delivered at the investigation centre to potential participants during a pre-screen visit. Participants were then informed that the impact of MC on the acquisition of sexually transmitted infections (STIs), including HIV, is not known. A minimum of 3 d after the pre-screen visit, potential participants were screened for eligibility. Potential participants with genital ulcerations were temporarily excluded until successful treatment. The inclusion and exclusion criteria are listed in Table 1. The participants received a total of 300 South African Rand as compensation (1 South African Rand ~ 0.12 Euro). The protocol, the consent form, and the participant information sheet are provided as Text S1–S3.

Randomization

At the end of the screen visit, following screening and written consent, participants were divided into two groups, using sealed envelopes. Participants requested to participate actively in the random assignment. Consequently, each participant was invited by the manager of the centre to choose an envelope containing the group name from a basket of ten envelopes. After each randomization, a new envelope was added to the basket. This added envelope was taken sequentially from a set of envelopes pre-prepared in such a way that each set of envelopes contained five for the "Control" and five for the "Intervention" arm. Participants of the intervention group were offered to be circumcised within a week. Participants of the control group were asked to wait until the end of the trial before being offered to be circumcised.

Follow-Up and Data Collection

After the screen visit, which took place at month 1 (M1), the three follow-up visits took place at the end of M3, M12, and M21. The M3 visit was designed to study the possible impact of surgery on HIV acquisition as a result of sexual activity during the healing phase following circumcision or contamination during surgery. These three follow-up visits defined three sequential periods, M1–M3, M4–M12, and M13–M21, with expected durations of 3, 9, and 9 mo, respectively. The duration of these periods was measured in days from the dates of the visits, the day after the end of a period being the beginning of the next period.

A participant lost to follow-up was defined as a participant

Table 1. Inclusion and Exclusion Criteria

Type	Criterion
Inclusion criteria	To be a male between the age of 18 and 24
	To wish to be circumcised
	To reside in the Orange Farm area or surrounding areas
	To be able to understand the nature of the trial
	To agree to be randomized to either of the two groups (the intervention group and the control group)
	To agree to come to three follow-up visits
	To agree to answer general health questions and questions related to sexual activity
	To agree to have genital examinations
	To agree to give blood samples tested for HIV and syphilis
Exclusion criteria	To be circumcised
	To have had any contraindication to MC

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who had not completed a planned visit in the 2 mo following the planned date of this visit and who did not complete any further visit. A missing visit was defined as a visit not completed prior to a completed visit.

At each of the four visits, each participant was invited to answer a face-to-face questionnaire, to provide a blood sample, and to have a genital examination and an individual counselling session. The questionnaire allowed for collection of data on background characteristics and reported sexual behaviour. The last section of the questionnaire allowed for the description of all sexual partnerships over the previous 3 mo for the M3 visit and over the previous 12 mo for all other visits. This section allowed each participant to describe the number of sexual contacts, the date of first and last sexual contact, the frequency of condom use (never, sometimes, always), and the type of partnership (spousal or non-spousal), a spousal partner being defined as a sexual partner with whom the respondent is married or living as married. Characteristics of sexual behaviour during the 9-mo periods M4–M12 and M13–M21 were determined from this section, using the dates of first and last sexual contact of each sexual partner. The genital examination was performed by a trained nurse who recorded the circumcision status and took a blood sample from each participant. Blood samples were tested for syphilis and HIV-1.

The counselling session (15–20 min) was delivered by a certified counsellor and focused on information about STIs in general and HIV in particular and on how to prevent the risk of infection. During this session, participants were encouraged to attend voluntary counselling and testing in a public clinic located 200 m away from the investigation centre or in a voluntary counselling and testing (VCT) centre funded by the project and located in the same building as the investigation centre. Condoms were provided in the waiting room of the investigation centre and were also provided by the counsellor. Participants who had symptoms of STIs, as assessed by the nurse during the genital examination, or who tested positive for syphilis were treated at the local clinic or by doctors working for the project. A specific programme for prevention of opportunistic infections and delivery of antiretroviral treatment, if required, was put in place at the VCT centre to assist participants who attended VCT and who tested positive for HIV. The arrangement will remain in place until the public sector programme becomes operational in the area.

The standard of care in South Africa at the beginning of the trial in July 2002 included VCT but not access to antiretroviral therapy. With the formal introduction of access to antiretroviral therapy in 2004, there were increased efforts to encourage participants to attend VCT and referrals to appropriate facilities were instituted. In this context, it was decided to include participants independent of VCT attendance. Consideration for making HIV testing compulsory for participation in the trial or recruiting only those who tested HIV-negative would certainly lead to stigmatization, and the investigators considered that the whole concept of VCT was that it should be voluntary. They considered it unethical to inform participants of their HIV status without their permission, even if they thought that participants should be aware of their HIV status. They also considered it unethical to deter from participating in the study potentially at-risk men who did not want to know their HIV status. Indeed, HIV-positive participants would benefit from the trial: (a) by receiving counselling at each visit, (b) by undergoing clinical examination and syphilis testing, and (c) by having a medicalized circumcision that could possibly protect them or their sexual partners against other STIs or even against re-infection by HIV.

Male Circumcision

The median (interquartile range [IQR]) duration between randomization and MC was one day (0–2). The circumcisions were performed by three local general practitioners in their surgical offices. The general practitioners were experienced in MC practices. The cost of each circumcision was 300 South African Rand and was paid for by the project. The procedure was standardized and used the forceps-guided method, as is widely practiced in South Africa, and was reviewed by the Department of Urology, University of the Witwatersrand Medical School, South Africa.

Quality of the Data, Blinding, Confidentiality, and Data Management

To ensure confidentiality, participants' files were kept in a locked room at the centre and each participant received a number that was used to identify all documents related to that person. To ensure blinding of study personnel, the randomization group information was not available to the personnel in charge of counselling or collecting information in the centre during the participants' visits.

Questionnaires were checked at the end of each interview. Participants failing to turn up for any follow-up visit were visited at home by trial staff, who encouraged them to come for the follow-up visits or ascertained the reasons for dropping out.

Laboratory results were stored in a database that was independent of the one used to store the information related to each participant. During the study, no HIV results were available to the investigation centre or to the investigators, apart from the statistician in charge of the interim analysis.

Laboratory results and data collected from questionnaires were entered twice in a database (Microsoft Access, Redmond, Washington, United States) by different people. The two entries were compared, and discrepancies were corrected. The data were then re-checked for inconsistencies using the source documents. After the data had been cleaned, they were imported into the statistical package SPSS for Windows version 8 (SPSS, Chicago, Illinois, United States) and R (version 2.0.1) for analysis [17].

Laboratory Procedures

Following the interview, a trained nurse collected whole blood samples in the investigation centre. One EDTA blood tube of 10 ml of venous blood was taken and immediately centrifuged at 400 g for 10 min, and five aliquots of plasma were frozen at −20 °C. The samples were identified only by the participant number and transported each week to the laboratory, where they were stored at −70 °C and tested.

An ELISA screen (Genscreen HIV1/2 version 2, Bio-Rad, France) and two ELISA confirmatory tests (Wellcozyme HIV recombinant, Abbott Murex, Dartford, United Kingdom, and Vironostika HIV Uni-Formm II plus O, bioMérieux, Boxtel, Netherlands) were used to test plasma for HIV-1 infection. Samples that were positive on all three ELISAs were regarded as "positive" and all others as "negative" [18].

Ethics

The research protocol was reviewed and approved by the University of Witwatersrand Human Research Ethics Committee (Medical) on 22 February 2002 (protocol study no. M020104). The trial was also approved by the Scientific Commission of the French National Agency for AIDS Research (ANRS; protocol study no. 1265; 2002, decision No. 50) and obtained authorization from the City of Johannesburg, Region 11, on 25 February 2002. A Data and Safety Monitoring Board was responsible for analyzing adverse events and for deciding on the results of the interim analysis.

Adverse Events

Adverse events (AEs) were documented and analyzed for all participants, including those who were HIV-positive at randomization. These AEs related to surgery, and that occurred in the first month post-surgery, were reported by the practitioners using a specific form. In addition, at each visit to the centre the nurse completed a questionnaire after the genital examination to record adverse events. During home visits for missing participants, any deaths were recorded.

Sample Size and Interim Analysis

The total sample size was initially calculated to be 2,580 HIV-negative participants in order to obtain a power of 80% to detect a 50% reduction in the proportion of HIV infection between the groups at a 5% significance level, assuming an HIV incidence of 2.2 per 100 person-years (py) in the control group. This number, calculated using Fisher's exact test, was increased to 3,035 to account for 15% of participants lost to follow-up. An interim analysis was planned for when all the M12 visits had been completed, and this was conducted blind with the database obtained on 29 November 2004. At the time of the interim analysis, the total follow-up included an estimated 63% of the total number of py that would have been collected at the end of the study, leading to a threshold value of 0.0095, as determined by the Lan-DeMets alpha-spending function method [19].

Statistical Analysis

While participants with a HIV-positive test at M1 were followed in the same way as the other participants, they were excluded from the statistical analysis. HIV status was considered as censored data with time being continuous, observed in a grouped form (at the end of each period), with non-uniform duration of periods. These data were modelled using a piecewise exponential, proportional hazards model in which the baseline hazard is constant in each period. This theoretical model allows the precise duration between each visit and time-dependent covariate to be taken into account. It was implemented by running a Poisson log-linear model on a dataset composed of lines corresponding to the periods M1–M3, M4–M12, and M13–M21, in which the participant stayed HIV-negative or became HIV-positive [20–22]. Consequently, in this dataset, each individual was represented by a maximum of three lines. This type of model gives an incidence rate and incidence rate ratio (RR) of HIV infection among men of the intervention group in comparison with men of the control group. The protection against HIV infection was calculated as $1 - RR$.

At the interim analysis, the RR was 0.37 in the intervention group, as compared with the control group, with a p value of 0.00073, below the threshold value. The Data and Safety Monitoring Board advised the investigators to interrupt the trial and offer circumcision to the control group, who were then asked to come to the investigation centre, where MC was advised and proposed. The database corresponding to planned visits up to 30 April 2005 was then analyzed, and the results are presented in this paper. Because the study was interrupted, some participants did not have a full follow-up on that date, and their visits that were not yet completed are described as "planned" in this article.

Adjusted rates and RRs were obtained by taking into account covariates that were calculated for each period when they were time-dependent. Three nested models were developed. The model-1 included the period number, which was included as categorical variables, with the logarithm of the duration of exposure in each period in days as an offset. In the model-2, the calendar period of recruiting and background characteristics of the participants were added. In the model-3, behavioural time-dependent covariates, characterizing the behaviour of participants during each period, were also added.

The background characteristics of the participants considered were age (less than or equal to 21 y, more than 21 y), religion (Catholic or Protestant, African traditional, other), ethnic group (Zulu, Sotho, other), and alcohol consumption in the past month. The five reported sexual behaviour covariates considered were, for each period of follow-up, being at-risk behaviour (defined as having at least one sexual contact unprotected by condom), having a spousal partner, the number of non-spousal sexual partners, the number of sexual contacts, having at least one relationship with only one sexual contact. In addition, health-seeking behaviour was characterized by at least one visit to a clinic for a genital problem during the 12-mo period prior to a visit to the centre.

Additional analyses were also performed. (a) The impact of the intervention was assessed among those having completed their M21 visit. (b) The impact of the intervention on participants who were 1 mo or more late to at least one follow-up visit or missed one follow-up visit was compared with the impact of the intervention on other participants by testing the corresponding interaction term between this factor and the randomization group. (c) To analyze the impact of the 6-wk period of abstinence, the analysis was repeated with the duration of the period M1–M3 reduced by 42 d in the intervention group. Forty-two days was the median (IQR = 28–56) interval between MC and first sexual contact reported by sexually experienced participants of the intervention group. (d) The effects of MC across the ethnic groups were studied by assessing this impact among the two major ethnic groups of this study (Zulus and Sothos) and by testing the corresponding interaction term. (e) Finally, while all analyses were performed in intention-to-treat, a per-protocol analysis was performed using the circumcision status observed at each visit.

Six comparisons of the behavioural factors for each of the periods M4–M12 and M13–M21 were performed. Independence of behavioural categorical data between the randomization groups was tested using Fisher's exact test, and the Kruskal-Wallis test was used for quantitative behavioural

Table 2. Baseline Characteristics of HIV-Negative Men Enrolled in the Trial

Background Characteristics		Control $n = 1,582$	Intervention $n = 1,546$
Age	Less than or equal to 21 y	52.4%	48.6%
	More than 21 y	47.6%	51.4%
Primary level of education completed		98.4%	98.3%
Religion	African traditional	47.0%	51.6%
	Protestant or Catholic	11.1%	11.9%
	Other religion	41.8%	36.5%
Ethnic group	Sotho	47.3%	49.0%
	Zulu	38.1%	32.8%
	Other	14.6%	18.2%
Drank alcohol in the past month		41.9%	42.2%
Reported sexual behaviour			
Have had first sexual experience		90.5%	91.8%
Median (IQR) age at first sex (years)[a]		16.6 (15.2–18.4)	16.8 (15.4–18.5)
Median (IQR) number of lifetime sex partners[b]		4 (2–7)	4 (3–7)
Used a condom at first sex[b]		13.4%	15.2%
Ever used a condom[b]		81.2%	82.3%
At-risk behaviour[c,d]		46.7%	46.8%
Married or living as married[d]		1.8%	1.8%
Mean (IQR) number of non-spousal partners[e]		1.4 (0–2)	1.4 (0–2)
At least one sexual partnership with only one sexual contact[e]		29.8%	30.7%
Mean (IQR) number of sexual contacts[e]		8.0 (0–8)	8.7 (1–8)
Attended a clinic for a health problem related to the genital area[e]		10.0%	9.6%

[a] Calculated using censored data analysis
[b] Among those having had first sexual experience
[c] Defined as having at least one sexual contact not protected by condom
[d] At some time during the past 12 mo before randomization
[e] During the past 12 mo before randomization
IQR, interquartile range
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variables. Assuming that these comparisons were independent, and to keep the overall risk of type I error equal to 0.05, the level of significance was set as $1.00 - 0.95^{1/6} = 0.0085$.

Results

Table 2 gives the baseline characteristics for the HIV-negative participants. The median age (IQR) was 21.0 y (19.6–22.5). Most of the participants had completed the primary level of education. Very few were married or living as married, and about half were at-risk behaviour. Figure 1 shows the trial flowchart. A total of 3,274 men participated in the trial. There were 146 (prevalence 4.5%) HIV-positive participants at randomization. The difference in size between the intervention and control group was 34 (1,620 versus 1,654).

Among the 3,128 HIV-negative participants at randomization, the visits at M3, M12, and M21 took place at (median; IQR) 3.0 (3.0–3.2), 12.0 (11.9–12.1), and 20.9 mo (20.9–21.2) after randomization, respectively. The mean (IQR) follow-up was 18.1 mo (13.0–21.0).

The fraction of participants lost to follow-up was 8.0 % (251/3128), with 6.5% (100/1546) in the intervention group and 9.5% (151/1582) in the control groups ($p = 0.0016$, Fisher's exact test). Among the participants lost to follow-up at the visit M12 or M21, none (0/124) were HIV-positive at their previous completed visit.

During the study, 20 and 49 participants acquired HIV infection in the intervention and control groups, respectively, corresponding to incidence rates (95% CI) of 0.85 per 100 py (0.55–1.32) and 2.1 per 100 py (1.6–2.8) in the intervention and control groups, respectively. Using model-1, the RR of HIV infection for the intervention group in comparison with the control group was 0.40 (0.24–0.68), $p = 0.00059$ (Table 3). This RR corresponds to a protection of 60% (32–76) against HIV infection. This result is equivalent to saying that during the period M1–M21 the intervention prevented six out of ten potential infections.

When considering only those participants who completed their M21 visit, the RR was 0.38 (0.22–0.67), $p < 0.001$. In comparison with the others, those who were 1 mo or more late to at least one follow-up visit or missed one follow-up visit (1178/3128; 37.7%) had the same risk of HIV infection (RR = 1.06; 0.65–1.73; $p = 0.82$) and were not differently protected by MC ($p = 0.69$). When reducing the M1–M3 period by 42 d in the intervention group, the RR was RR = 0.43 (0.26–0.73), $p = 0.0016$, a value close to the RR obtained in the intention-to-treat analysis. This indicates that the 6-wk period of abstinence plays a minor role in explaining the effect of the intervention during the period M1–M21. Among the two major ethnic groups of the participants, Zulus ($n = 1,109$) and Sothos ($n = 1,506$), the RR was 0.60 (0.25–1.41), $p = 0.24$, and 0.42 (0.20–0.88), $p = 0.022$, respectively. These two RRs were not significantly different ($p = 0.55$). The per-protocol analysis gave RR = 0.24 (0.14–0.44), $p < 0.001$, a value lower to the RR obtained in the intention-to-treat analysis. The difference of the results given by the two analyses is at least partly explained by the cross-overs. In the intervention group, 6.5% (93/1432) were not circumcised at M3, and in the control group, 10.3% (114/1105) were circumcised at M21 (Figure 1).

For the periods M1–M3, M4–M12, and M13–M21, the number of HIV infections was two, seven, and 11 in the

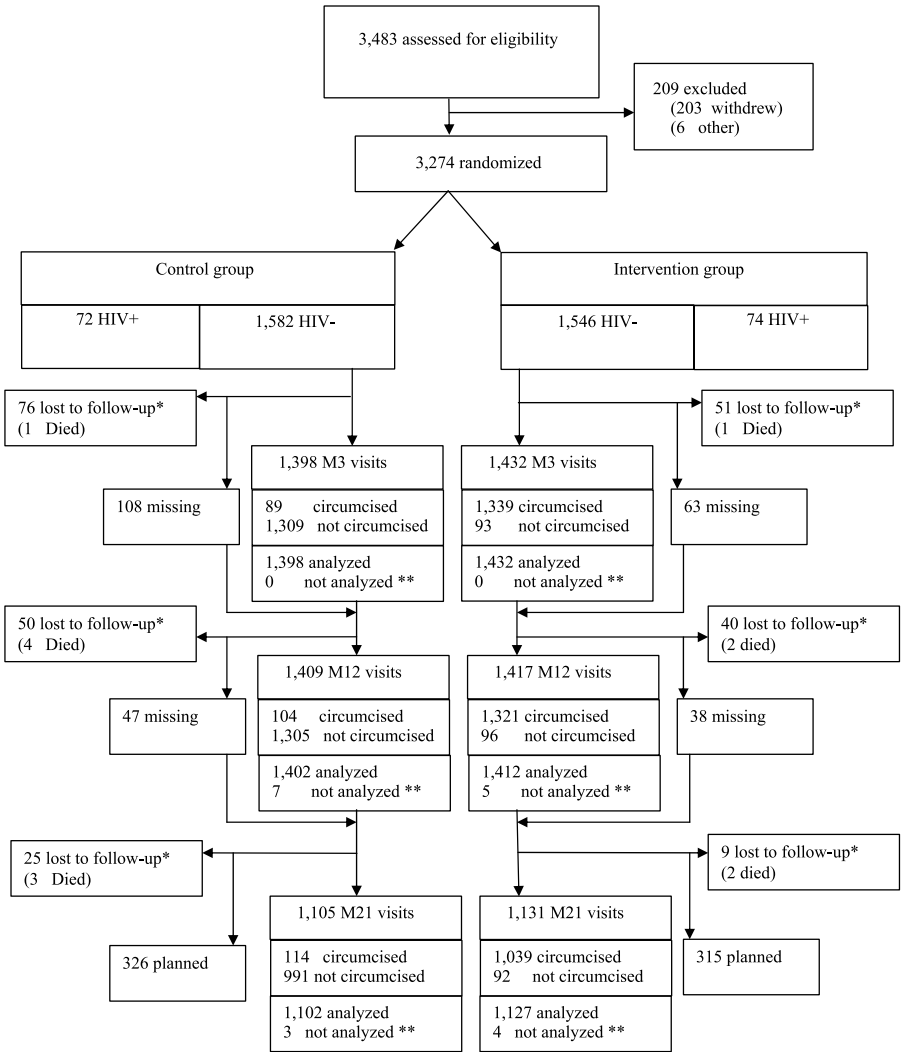

Figure 1. Trial Profile

This figure describes the state of the trial corresponding to planned visits up to 30 April 2005. HIV-positive and HIV-negative participants were randomized. All were followed, but only participants HIV-negative at randomization were analyzed and are represented in the three follow-up visits of the figure. After randomization, the participants could attend the 3-mo visit, miss it, or be excluded from follow-up (death or loss to follow-up). The non-excluded participants who attended the 3-mo visit could then attend the 12-mo visit, miss it, or be excluded (death or loss to follow-up). The non-excluded participants of the 12-mo visit could then attend the 21-mo visit, be excluded (death or loss to follow-up) or were planning to attend the 21-mo visit but had not yet done so, because of the interruption of the trial.

*, did not come for the scheduled visit (refused, withdrew, moved away or died); **, no blood sample

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Table 3. Characteristics of the Follow-Up Period

Characteristic	Period[a]			
	M1–M3	M4–M12	M13–M21	M1–M21 (total)
Number of HIV infections	11	22	36	69
Follow-up (py)	881	2,159	1,652	4,693
Incidence rates percent py (95% CI)[b]	1.25 (0.69–2.26)	1.02 (0.67–1.55)	2.20 (1.59–3.05)	1.48 (1.17–1.87)
Incidence RRs (95% CI) of intervention versus control[b]	0.23 (0.05–1.04) $p = 0.057$	0.46 (0.19–1.13) $p = 0.091$	0.43 (0.21–0.87) $p = 0.019$	0.40 (0.24–0.68) $p = 0.00059$

[a] The follow-up periods are from M1–M3, M4–M12, and M13–M21.

[b] Obtained using a piecewise exponential, proportional hazards model, which was implemented with a Poisson log-linear model. Duration of exposure was the duration of each period for those staying HIV-negative and the duration of half the period for those becoming HIV-positive (model-1; see text).

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Table 4. Multivariate RRs of HIV Incidence

Categories of Factors	Factors	Values of Factors	HIV Cases	Follow-Up (py)	HIV Incidence Rates (95% CI; per 100 py)[a]	Incidence RRs (95% CI) of Intervention versus Control (95% CI)[a,b]
	Randomization group	Intervention	20	2,354	0.85 (0.55–1.32)	0.39 (0.23–0.66) $p = 0.00049$
		Control	49	2,339	2.11 (1.60–2.80)	1
	Recruitment period	After 30 December 2002	41	3,251	1.27 (0.93–1.72)	0.64 (0.39–1.06) $p = 0.081$
		At or before 30 December 2002	28	1,442	1.96 (1.35–2.84)	1
Individual characteristics	Age group	More than 21 y	46	2,284	2.03 (1.52–2.71)	1.99 (1.19–3.34) $p = 0.0086$
		Less than or equal to 21 y	23	2,408	0.96 (0.64–1.44)	1
	Religion	Catholic or Protestant	25	1,845	1.36 (0.92–2.02)	0.49 (0.19–1.25) $p = 0.14$
		Other	5	576	0.87 (0.36–2.09)	0.67 (0.40–1.12) $p = 0.12$
		African traditional	39	2,271	1.73 (1.26–2.37)	1
	Ethnic group	Zulu	13	772	1.70 (0.98–2.92)	0.83 (0.48–1.42) $p = 0.49$
		Other	23	1,689	1.37 (0.91–2.06)	1
		Sotho	33	2,232	1.49 (1.06–2.09)	1.04 (0.55–2.00) $p = 0.90$
	Drank alcohol in the previous month	Yes	35	1,954	1.80 (1.29–2.51)	1.29 (0.80–2.09) $p = 0.30$
		No	34	2,738	1.25 (0.89–1.75)	1
Behavioural factors	Being at risk behaviour[c,d]	Yes	46	2,498	1.86 (1.39–2.48)	1.02 (0.57–1.83) $p = 0.95$
		No	23	2,076	1.11 (0.74–1.67)	1
	Married or living as married[d]	Yes	4	185	2.19 (0.82–5.83)	0.68 (0.23–1.99) $p = 0.48$
		No	65	4,389	1.49 (1.17–1.90)	1
	Number of non-spousal partners[e]	> 1	14	817	1.73 (1.02–2.91)	0.91 (0.44–1.87) $p = 0.79$
		0–1	55	3,758	1.47 (1.13–1.92)	1
	At least one sexual partnership with only one sexual contact[d]	Yes	14	1,009	1.39 (0.83–2.36)	0.98 (0.49–1.96) $p = 0.96$
		No	55	3,555	1.55 (1.19–2.02)	1
	Number of sexual contacts[e]	> 5	29	1,207	2.43 (1.69–3.50)	1.61 (0.90–2.88) $p = 0.11$
		0–5	40	3,368	1.19 (0.87–1.63)	1
	Attended a clinic for a health problem related to the genitals[f]	Yes	21	276	7.84 (5.11–12.02)	5.73 (3.33–9.84) $p < 0.001$
		No	48	4,299	1.12 (0.85–1.49)	1

[a] Obtained using a piecewise exponential, proportional hazards model, which was implemented with a Poisson log-linear model. Duration of exposure was the duration of each period for those staying HIV-negative and the duration of half the period for those becoming HIV-positive.
[b] Adjusted for all variables indicated in the column (model-3; see text)
[c] See footnote c in Table 2
[d] At some time in the past 3-mo period before M3, and in the past 9-mo period before M12 and M21
[e] In the past 3-mo period before M3, and in the past 9-mo period before M12 and M21
[f] In the past 12 mo before each follow-up visit
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intervention group and nine, 15, and 25 in the control group. The RR for each of these periods is given in Table 3. In the period M1–M3, there was an RR of 0.23 close to the significance level, which was slightly higher when taking into account the 42 d of abstinence (RR = 0.37; 0.08–1.72; $p = 0.21$).

Using model-2, an RR was found similar to that obtained with model-1: 0.38 (0.23–0.65), $p < 0.001$. This result is attributable to the randomization process, which distributed the characteristics equally between the intervention and control groups.

Of the five reported sexual behavioural factors, all were higher in the intervention group than in the control group during the period M4–M12, and four out of five were higher during the period M13–M21. Only the mean number of sexual contacts showed statistically significant differences during the period M4–M12 (5.9 versus 5.0, $p < 0.001$) and during the period M13–M21 (7.5 versus 6.4, $p = 0.0015$). The proportion of participants attending a clinic for a genital problem in the 12 mo prior to M12 was lower in the intervention group than in the control group (4.7% versus 7.2%, $p = 0.0067$).

Using model-3, the RR, adjusted on behavioural characteristics, reported by participants during the follow-up is similar to the RR obtained with model-1 (Table 4). This last result indicates that the protective effect of the intervention is not attributable to the change of reported behaviour associated

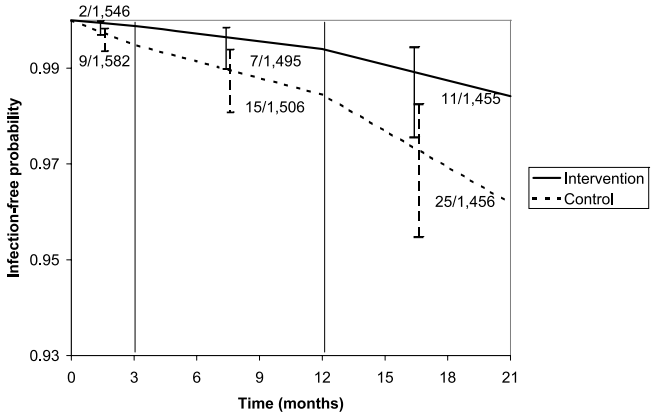

Figure 2. Infection-Free Probability As a Function of Time and of Randomization

This figure represents the infection-free probability using a piecewise exponential distribution with boundaries at M3, M12, and M21 obtained with a Poisson log-linear model (see text). Each segment of exponential has been fitted to the data in each period for each randomization group. The 95% confidence intervals have been represented in the middle of each period. x/y is the number of HIV infections observed in each period (x) and the number of persons at the beginning of the period (y).

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with the intervention and shows that adjustment for potential confounders has little effect on the association of MC and HIV incidence.

Figure 2 shows the fitted infection-free probability as a function of time and of randomization. Table 5 describes the 60 (3.8%) AEs that were reported during surgery or in the first month following surgery among 1,568 MCs performed in the intervention group, HIV-positive at randomization included. The proportion of AE was higher among those who were HIV-positive at randomization, and the difference is close to significance ($p = 0.056$, Fisher's exact test). At M3, 98.5% of those who were circumcised (HIV-positive at randomization included), were "very satisfied" with the result of the circumcision. Adverse events recorded at the end of the follow-up (M21) are described in Table 6.

Home visits for late participants revealed 16 deaths among participants (HIV-positive at randomization included), of whom six had been circumcised, but examination of death certificates, reports from doctors who carried out the MC, interviews with relatives, and timing of these deaths revealed no deaths related to MC. The mortality rate from the South African Census 2001 data [23] in the age groups 15–19 and 20–24 for the black population of the Gauteng province was 2.4 and 3.9 per 1,000 per year. These figures lead to an estimate of 3.5 per 1,000 per year at the mean age (21.0 y) of our participants. In turn, this value leads to an estimated number of deaths of 15.7 using the mean follow-up, which is close to the number of deaths observed in our trial.

Table 5. Adverse Events during Surgery or in the First Month following Surgery among Those Having Been Randomized in the Intervention Group, as a Function of HIV Status at Randomization

Adverse Event	HIV-Negative at Randomization (n = 1,495 MC)	HIV-Positive at Randomization (n = 73 MC)	Total (n = 1,568 MC)
Death	0 (0%)	0 (0%)	0 (0%)
Pain	12 (22.2%)	1 (16.7%)	13 (31.7%)
Excessive bleeding	9 (16.7%)	0 (0%)	9 (15%)
Infection	2 (3.7%)	1 (16.7%)	3 (5%)
Damage to the penis	3 (5.6%)	1 (16.7%)	4 (6.7%)
Swelling or haematoma	9 (16.7%)	1 (16.7%)	10 (16.7%)
Anaesthesia-related events	1 (1.9%)	0 (0%)	1 (1.7%)
Excessive skin removed	0 (0%)	0 (0%)	0 (0%)
Insufficient skin removed	4 (7.4%)	0 (0%)	4 (6.7%)
Delayed wound healing	1 (1.9%)	1 (16.7%)	2 (3.3%)
Problems with urinating	0 (0%)	0 (0%)	0 (0%)
Problems with appearance	8 (14.8%)	1 (16.7%)	9 (15%)
Other cause	5 (9.3%)	0	5 (8.3%)
Total	54 (100%) [3.6%]	6 (100%) [8.2%]	60 (100%) [3.8%]

Percentages of adverse events are given in parentheses, and percentages of MCs are given in brackets.

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Discussion

This study provides the first experimental evidence of the efficacy of MC in protecting men against HIV infection. It was conducted in a general population, and it is the first randomized control trial testing the impact on health of MC. The demonstration in this study of a causal association between HIV infection and MC is consistent with protection suggested by meta-analyses of observational studies [12] but

Table 6. Adverse Events at the End of the Follow-Up (M21) among Those Having Been Randomized in the Intervention Group, As a Function of HIV Status at Randomization

Adverse Event	HIV-Negative at Randomization (n = 1,131 M21 visits)	HIV-Positive at Randomization (n = 54 M21 visits)
Problem with urinating[a]	3 (27.3%)	0 (0%)
Dissatisfied with the appearance of the penis[a]	4 (36.4%)	0 (0%)
Mild or moderate erectile dysfunction[a]	4 (36.4%)	0 (0%)
Torsion of penis[b]	0 (0%)	0 (0%)
Total (%)	11 (100%) [1.0%]	0 (100%) [0%]

Percentages of adverse events are given in parentheses, and percentages of MC are given in brackets.

[a] Reported by participants

[b] Collected by a nurse

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with a higher protective effect. This difference can be explained, at least partly, by the effect of bias and confounding factors associated with cross-sectional studies. High values ranging from 0.12 to 0.29 of protective effect of MC have been reported in prospective studies conducted in high-risk groups [6,8–11]. Our study is also the first experimental study demonstrating that surgery can be used to prevent an infectious disease. In addition, this finding is an a posteriori proof of the use of MC to improve hygiene in the common meaning of not being infected.

This study has some limitations. It was conducted in one area in sub-Saharan Africa and, therefore, may not be generalizable to other places. Nevertheless, because of the similar route of transmission of HIV in sub-Saharan Africa and because observational studies from various areas of sub-Saharan Africa have shown an association between HIV status and MC [12], the result of this trial is applicable to all of sub-Saharan Africa with some degree of confidence.

Even though some participants were lost during the follow-up, and the loss to follow-up rate was greater than the event rate, the impact of missing participants on the overall results of this study is likely to be small not only because the loss to follow-up was small for a cohort study conducted in a general population, but also because those who were late for at least one follow-up visit were protected by MC just as the other participants. The reason for this loss to follow-up was a result of participants moving from the area or being unreachable, and not a result of HIV infection.

Because the Data and Safety Monitoring Board recommended to stop the trial after the intermediate analysis, it was not possible to follow all the participants as initially planned, and, as a consequence, only those participants recruited at the beginning had a full follow-up. This potential bias was taken into account by adjusting the analysis for the recruitment period; such an adjustment cannot fully account for the confounding effect associated with partial follow-up. When restricting the analysis to those participants who had a full follow-up, the intervention had an effect that was similar in size and significance, suggesting that this potential bias had a negligible impact.

A specific survey was implemented after the end of the recruiting period in order to assess the satisfaction of the results of the randomization. Of the participants, 65.3% said they were happy. However, the results also showed that a limited number of participants (7.5%), strongly unhappy with their group of randomization, were allocated and recorded in the other group. They were analyzed in their randomization group in the intention-to-treat analysis. The findings were confirmed by the person in charge of randomization. This factor contributed to increase the cross-over, which remained low, and to dilute the measure of the effect of the intervention, which remained high.

Another limitation concerns the timescale of this study. Participants were followed up for a short period of time, and, therefore, this study did not explore the long-term protective effect of MC.

The protective effect of MC on HIV infection was unchanged when controlling for sexual behaviour, including condom use, which was taken into account when defining those at-risk behaviour, the period of abstinence in the intervention group following MC, and heath-seeking behaviour, which was considered because treatment of STIs can have an effect on HIV acquisition [24]. This shows that these factors play a minor role in explaining the protective effect of MC on HIV infection. The reasons for this protective effect of MC on HIV acquisition have to be found elsewhere, and several direct or indirect factors may explain this [25]. Direct factors may be keratinization of the glans when not protected by the foreskin, short drying after sexual contact, reducing the life expectancy of HIV on the penis after sexual contact with an HIV-positive partner, reduction of the total surface of the skin of the penis, and reduction of target cells, which are numerous on the foreskin [26]. Indirect factors may be a reduction in acquisition of other STIs, which in turn will reduce the acquisition of HIV. Our study does not allow for identification of the mechanism(s) of the protective effect of MC on HIV acquisition.

The first and obvious consequence of this study is that MC should be recognized as an important means to reduce the risk of males becoming infected by HIV. As shown by our study, MC is useful and feasible even among sexually experienced men living in an area with high HIV prevalence. Indeed, in our study the intervention delivered by local general practitioners resulted in a limited and reasonable number of adverse events and did not lead to an increase in deaths. In addition to the protective role in men, MC will indirectly protect women and, therefore, children from HIV infection because if men are less susceptible to HIV acquisition, women will be less exposed. Moreover, MC may also be protective against male-to-female HIV transmission, but this will require further investigation [7]. The role that women can play in promoting MC is potentially important. If women are aware of the protective effect of MC, this awareness could, in turn, have an impact on the prevalence of MC by encouraging males to become circumcised.

It was found that the protective effect of MC is high. MC provides a degree of protection against acquiring HIV infection equivalent to what a vaccine of high efficacy would have achieved. Consequently, the authors think that MC should be regarded as an important public health intervention for preventing the spread of HIV. MC could be incorporated rapidly into the national plans of countries where most males are not circumcised and where the spread of HIV is mainly heterosexual. This is even more important at a time when no vaccine or microbicides are currently available and when delivering antiretroviral treatments under WHO guidelines will have only a small impact on the spread of HIV [27]. In addition, MC is an inexpensive means of prevention, performed only once, and men can be circumcised over a wide age range, from childhood to adulthood.

The potential impact of prevention programmes based on MC is difficult to assess at population level and requires modelling. From the results of this study and of the meta-analysis quoted above, it can be predicted that widespread MC could lead to a strong reduction of the spread of HIV. The availability of a simple and ancient practice with a high potential effect on the spread of HIV is remarkable and should encourage decision makers to take MC into consideration as policy. Because most of southern and East Africa is concerned, the number of HIV infections that could be avoided by the widespread implementation of MC is high.

There are potential risks in promoting MC as way of reducing the risk of HIV infection. MC can be performed under poor hygienic conditions, leading to not only infection,

bleeding, and permanent injury, but also HIV infection from non-sterilized instruments, and possible death if appropriate treatment of sequelae is not provided. In the healing period, sexually active men are likely to be at a higher risk of HIV infection, and this risk should not be underestimated. MC does not provide full protection and, if perceived as full protection, could lead to reduction of protection of men who, for example, decrease their condom use or otherwise engage in riskier behaviour. It was found that the intervention group had significantly more sexual contacts. While the protective effect of circumcision remained despite this increased risk, this should be a concern when considering implementation of circumcision as a means of preventing HIV infection. Finally, there is the danger of confusing MC with female circumcision, and that promotion of MC could be used by defenders of female circumcision to defend this practice.

Acceptability studies of the use of MC as a prevention measure against the spread of HIV have been conducted in South Africa [16,28], Kenya [29,30], Zimbabwe [31], and Botswana [32]. These studies, in which most of the uncircumcised African men expressed interest in becoming circumcised if performed safely and affordably, highlighted the potential of MC as a population-level intervention to reduce HIV spread. MC is a not a universal cultural practice, and cultural practices can be barriers in policy considerations. However, there are examples showing that the prevalence of MC can be changed. For example, in South Korea 50 years ago, almost no men were circumcised; today some 85% of Korean men 16–29 y old are circumcised [33].

The experimental demonstration of the protective effect of MC on the acquisition of HIV emphasizes the role of MC in explaining the heterogeneity of HIV prevalence in sub-Saharan Africa. From a multi-site study conducted in four African countries, MC, together with sexual behaviour, has been posited as an important factor in the heterogeneity of HIV prevalence in sub-Saharan Africa [34]. This role is confirmed and reinforced by the findings of the present study.

Supporting Information

Text S1. Effect of Medicalized Male Circumcision on the Incidence of HIV, Herpes Simplex Virus 2, and Genital Ulcerations

Found at DOI: 10.1371/journal.pmed.0020298.sd001 (204 KB PDF).

Text S2. Consent Form

Found at DOI: 10.1371/journal.pmed.0020298.sd002 (29 KB PDF).

Text S3. Participant Information Sheet

Found at DOI: 10.1371/journal.pmed.0020298.sd003 (45 KB PDF).

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Data Safety Monitoring Board: Peter Cleaton-Jones, Mohamed Haffejee (University of Witwatersrand, South Africa), and Jonathan Levin (MRC, South Africa)

This trial has been registered in http://www.clinicaltrials.gov under the number NCT00122525.

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Patient Summary

Background HIV/AIDS is one of the greatest threats to health worldwide. More than 3 million people died of AIDS last year, and about 5 million others became infected with HIV, bringing the total number of people living with the infection to nearly 40 million. The situation is particularly severe in Africa, which has 10% of the world's population but two-thirds of the world's people with HIV. In many African tribal groups, men are circumcised, usually in late childhood or early adolescence, and this is an important part of their cultural identity. In other African ethnic groups, men are not circumcised. By the late 1980s, researchers noticed that HIV infection rates were lower in those tribes where men were circumcised. But it was not clear whether it was circumcision itself or some other difference in behaviour between the circumcised and uncircumcised groups that gave some protection to the circumcised men against getting HIV.

What Did The Researchers Do? The researchers wanted to find out whether circumcising men could reduce their chance of becoming infected by HIV. They offered young, sexually active, heterosexual, uncircumcised men in Johannesburg, South Africa, the chance to have the operation. They explained that half of those who came forward would be circumcised right away (the "treatment group") and the other half would be circumcised 21 months later (the "control group"). Some 3,000 men joined the study. The group that each man was put into was decided at random. The plan was that all the men would visit the research clinic four times during this 21-month period, and that they would be tested for HIV each time. However, after 14 months, the number of new infections in the control group (49) was so much greater than the number in the treatment group (20) that it was considered unethical to continue the study. (The men in the control group were told they could be circumcised without any further delay.)

What Do These Findings Mean? Infections were 60% fewer in the treatment group, which seems to indicate that circumcised men are much less likely to become infected with HIV when having sex with infected women. In communities where HIV is common, circumcision may prove to be a valuable tool for reducing men's risk of getting infected. However, as with most studies, criticisms could be made of some aspects of the methods used, and more research is needed before we can be sure. We must also remember that circumcised men can still become infected, even though the risk might be lower. They should still take other steps to prevent themselves from getting HIV.

Where Can I Get More Information Online? The United Nations health agencies, including the WHO and UNAIDS, issued a statement when this research was first presented at a meeting in Brazil in July 2005:
http://www.who.int/mediacentre/news/releases/2005/pr32/en/
UNAIDS (http://www.unaids.org) has information about the state of the HIV/AIDS epidemic and prevention strategies. It produces an annual report and has documents on a wide range of topics. The Q&A documents are particularly useful:
http://www.unaids.org/EN/resources/questions_answers.asp#II
Many organizations provide information on AIDS prevention—for example, the Terrence Higgins Trust:
http://www.tht.org.uk
AEGIS is the world's largest searchable database on HIV and AIDS:
http://www.aegis.com

Male circumcision for HIV prevention in men in Rakai, Uganda: a randomised trial

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Summary

Background **Ecological and observational studies suggest that male circumcision reduces the risk of HIV acquisition in men. Our aim was to investigate the effect of male circumcision on HIV incidence in men.**

Methods **4996 uncircumcised, HIV-negative men aged 15–49 years who agreed to HIV testing and counselling were enrolled in this randomised trial in rural Rakai district, Uganda. Men were randomly assigned to receive immediate circumcision (n=2474) or circumcision delayed for 24 months (2522). HIV testing, physical examination, and interviews were repeated at 6, 12, and 24 month follow-up visits. The primary outcome was HIV incidence. Analyses were done on a modified intention-to-treat basis. This trial is registered with ClinicalTrials.gov, with the number NCT00425984.**

Findings **Baseline characteristics of the men in the intervention and control groups were much the same at enrolment. Retention rates were much the same in the two groups, with 90–92% of participants retained at all time points. In the modified intention-to-treat analysis, HIV incidence over 24 months was 0·66 cases per 100 person-years in the intervention group and 1·33 cases per 100 person-years in the control group (estimated efficacy of intervention 51%, 95% CI 16–72; p=0·006). The as-treated efficacy was 55% (95% CI 22–75; p=0·002); efficacy from the Kaplan-Meier time-to-HIV-detection as-treated analysis was 60% (30–77; p=0·003). HIV incidence was lower in the intervention group than it was in the control group in all sociodemographic, behavioural, and sexually transmitted disease symptom subgroups. Moderate or severe adverse events occurred in 84 (3·6%) circumcisions; all resolved with treatment. Behaviours were much the same in both groups during follow-up.**

Interpretation **Male circumcision reduced HIV incidence in men without behavioural disinhibition. Circumcision can be recommended for HIV prevention in men.**

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Introduction

A number of ecological and observational studies, mainly from sub-Saharan Africa, have suggested that male circumcision reduces the risk of HIV infection in men.[1–5] A meta-analysis of cross-sectional and prospective studies estimated that the adjusted summary rate ratio of male HIV acquisition associated with circumcision in general populations was 0·56 (95% CI 0·44–0·70); in high-risk populations the adjusted summary rate ratio was 0·29 (0·20–0·41).[1] However, observational findings do not consistently show protective associations in all studies, and to exclude the possibility of confounding due to differences in sexual risk behaviours and cultural or religious practices associated with circumcision is difficult. Thus, the potential efficacy of circumcision for HIV prevention can be determined only by randomised trials. One randomised trial done in South Africa was ended early after an interim analysis showed that circumcision reduced HIV incidence by 60% (32–76).[6] Two other randomised trials, one in Kisumu, Kenya and the other in Rakai, Uganda—the results of which we report here—were also stopped early on December 12, 2006, after interim analyses showed significant efficacy.

Methods

Patients

Our aim was to enrol 5000 HIV-negative, uncircumcised men aged 15–49 years who agreed to receive their HIV results through voluntary counselling and HIV testing provided by the study, and who consented to be randomly assigned to receive circumcision within about 2 weeks of enrolment (intervention group), or to have circumcision delayed for 24 months (control group). Screening and enrolment was done in a central study facility and in mobile facilities in the rural communities. Before screening, participants were informed of study procedures and risks through verbal presentations, written materials, and an information video. After providing written informed consent for screening, a venous blood sample was obtained for HIV testing, and participants were given a physical examination. Men who had contraindications for surgery (eg, anaemia, active genital infection, or other health risks) were treated, and if their medical condition resolved, they were re-screened and were enrolled into the trial if eligible. Those with anatomical abnormalities (eg, hypospadias) were excluded and referred to the urologist (SW) for management. Men who had medical indications for surgery (eg, severe phimosis) were excluded from the

trial and were offered circumcision as a service. Men who were HIV positive or declined to receive their HIV results were enrolled in a complementary trial that will be reported separately.

Eligible participants were asked to provide an additional written informed consent for enrolment. The consent forms described the risks and benefits of participation, randomisation, and other trial procedures, and provided information on HIV prevention (sexual abstinence, monogamous relationships with an uninfected partner, or consistent condom use). At enrolment, participants completed a detailed questionnaire administered by a trained interviewer on sociodemographic characteristics, sexual risk behaviours, genital hygiene, and health. Participants were asked to provide a urine sample for future testing of sexually transmitted infections. Two subpreputial and shaft swabs were also obtained for future testing for human papillomavirus infection and other sexually transmitted infections.

Procedures

Participants were randomly assigned to the intervention or control groups as follows. Treatment assignment was randomly generated in blocks of 20, stratified on community, with each community receiving four blocks of 20 assignment envelopes. Because enrolment occurred concurrently at more than one community site, this procedure ensured balance within sites. 20 assignments in opaque envelopes were placed in batches, and participants were asked to select one envelope from the box. After an assignment envelope was selected, it was replaced by the next envelope from the next batch designated for that community. This procedure could and did result in some temporary imbalance between study groups, with a maximum potential run of 20 instead of the standard ten same-group assignments, but it ensured that all participants had the opportunity to select one of 20 envelopes. An alternative procedure was considered in which participants would select from each block of 20 envelopes without replacement, which would ensure that every 20 assignments within a site was perfectly balanced. However, this method was rejected because it would progressively reduce a participant's options for envelope selection.

HIV status at screening was assessed by two enzyme immunoassays: Vironostika HIV-1 (Organon Teknika, Charlotte, NC, USA) and Welcozyme HIV 1+2 (Murex Diagnostics, Dartford, UK). Men with concordant negative results were enrolled into the trial. Discordant results were confirmed by western blot (Cambridge Biotech HIV-1 western blot, Caltype Biomedical Corp, Rockville, MD, USA); men who were negative by western blot were enrolled.

Men randomly assigned to the intervention group were asked to provide written consent for surgery on the day of the procedure, and were again provided with detailed information on the procedure, postoperative wound care, and the need to abstain from intercourse until complete wound healing had been certified by a clinical officer (equivalent to a physician's assistant). Participants were offered an information sheet to share with their wives or partners, explaining wound care, hygiene, and the need to abstain from intercourse until wound healing was complete. Surgery was provided within 2 weeks of enrolment to 2255 (91%) of the men in the intervention group; the median interval from enrolment to surgery was 2 days and the maximum delay was 149 days.

Circumcisions were done by trained and certified physicians in well-equipped operating theatres with careful attention to asepsis. All instruments, drapes, and other materials were autoclaved and sterility was assured by use of thermologues (Comply, 3M Healthcare, St Paul, MN, USA) and biological indicators (BT Sure, Barnsead/Thermolyne, Dubuque, IA, USA). Participants showered preoperatively to clean the genital area. The skin was prepared with povidone-iodine before administration of local anesthesia via a dorsal penile nerve block with a mixture of lidocaine and bupivacaine. Circumcision was done with the sleeve procedure, in which the foreskin was retracted and a distal incision made 0·5–1·0 cm proximal to the coronal sulcus, followed by a proximal incision on the unretracted prepuce at the corona. The superficial lamina of Bucks fascia was exposed and a sleeve of foreskin was freed from the underlying Bucks fascia and removed.[7] Bleeding was controlled with bipolar electrocautery and skin edges apposed with 4-0 absorbable sutures. Men were kept under observation for 30–60 minutes before discharge. Men who lived close to the surgical facility returned home, whereas those men who lived distant from the facility were offered free overnight accommodation in a study facility to ensure access to care should short-term complications arise.

Postoperative follow-up visits were scheduled at 24–48 hours, 5–9 days, and 4–6 weeks. The first visit was done at the surgical clinic site; subsequent visits occurred in mobile clinics in the communities. Care was available for participants at any time between scheduled visits. Follow-up was done by clinical officers who were trained by the urologist to diagnose and treat complications or to refer patients as needed. Potential adverse events related to surgery were predefined and graded as mild (requiring no treatment), moderate (requiring treatment), or severe complications (requiring surgical intervention [eg, wound exploration for active bleeding, repair of wound dehiscence], hospitalisation, or referral for specialised care). At each postoperative follow-up visit, participants were questioned about symptoms suggestive of complications, and the wound was inspected. Participants were asked about resumption of sexual intercourse, and those who had resumed such activity were asked about condom use.

All participants in both groups were followed up at 4–6 weeks, and at 6, 12, and 24 months post-enrolment. At each follow-up visit, participants answered questions on sexual risk behaviours (marital and non-marital

partners, condom use, alcohol consumption with sexual intercourse, and transactional sexual intercourse [ie, sexual intercourse in exchange for money or gifts]) and symptoms of sexually transmitted diseases (genital ulcer disease, urethral discharge, or dysuria) since their previous visit. Men were questioned about illnesses or hospitalisations to record all adverse events that occurred during trial participation. Additionally, men were examined to assess circumcision status and to diagnose any penile pathology. Samples of venous blood and urine and two penile swabs were collected, and repeat HIV counselling and testing and health education were provided. Free condoms were offered to all sexually active participants at all study visits, and were also available through community-based condom depots stocked by the Rakai programme.

The procedure for HIV testing at each follow-up visit was the same as at enrolment. All seroconversions or discordant enzyme immunoassay results were further assessed by western blot. For participants who had undergone seroconversion during follow-up, the previous serologically negative sample and in selected cases the first positive sample were tested by reverse transcriptase (RT) PCR (Amplicor HIV-1 Monitor version 1.5, Roche Molecular Systems, Branchburg, NJ, USA).

The Rakai Health Sciences Program has an HIV treatment programme that is funded by the Presidential Emergency Fund for AIDS Relief. Participants found to be HIV positive at trial screening and those who subsequently became infected with HIV during the trial were referred to the HIV treatment programme. All individuals enrolled into the HIV treatment programme were provided with prophylaxis with sulfamethoxazole-trimethoprim, insecticide-impregnated bednets, and water purification. Those who were eligible for antiretroviral therapy (CD4 cell count less than 250 cells per µL or WHO advanced stage III or stage IV disease) and who agreed to receive care were provided with antiretrovirals. None of the HIV-infected participants from the trial were eligible for antiretroviral therapy at the time of going to press.

The protocol was reviewed and approved by the Prevention Sciences Research Committee of the Division of AIDS, National Institute of Allergy and Infectious Diseases (NIAID), in the US National Institutes of Health (NIH), and by the Rakai community advisory board. The study was approved 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). The trial was done in accordance with the Good Clinical Practices and International Clinical Harmonisation guidelines with clinical trial monitoring done by Westat Corporation under a Division of AIDS, NIAID, NIH contract. The NIH Vaccine and Prevention Data Safety Monitoring Board oversaw the trial. Participants were compensated for their time, travel costs, and absence from work. Men received US$5 at screening and enrolment, $5 at the time of surgery, and $5 on completion of postoperative follow-up. Control participants who were circumcised at completion of their 24 months of follow-up received identical compensation. The amount of compensation for routine follow-up visits at 6, 12, and 24 months was $3 per visit. The community advisory board and institutional review boards approved this compensation as appropriate.

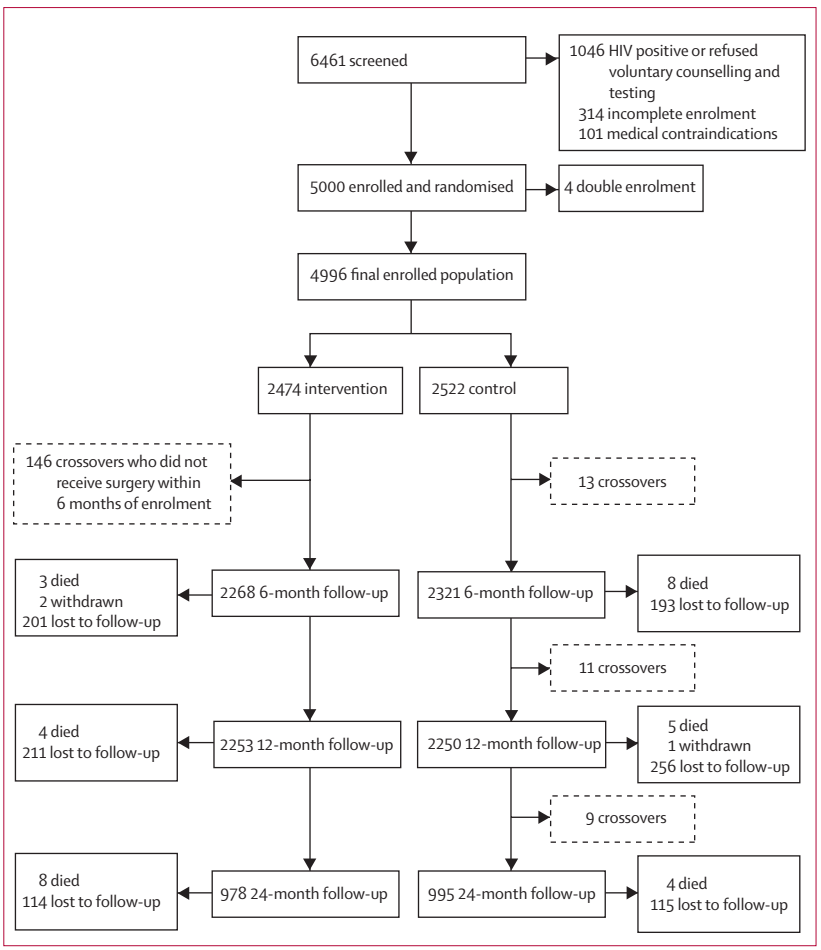

Figure 1: **Trial profile**

Statistical analysis

For incidence rate and Poisson regression calculations, HIV seroconversion was estimated assuming that

	Intervention group	Control group
6 months	2268/2469 (92%)	2321/2514 (92%)
12 months	2253/2464 (91%)	2250/2506 (90%)
24 months	978/1092 (90%)	995/1110 (90%)

Data are n/N (%). Percentages have been rounded.

Table 1: **Trial retention rates**

infection occurred at the mid-time point between the last negative and first positive serological tests, or at the time of the first positive RT-PCR for those participants seen during the period before HIV antibody seroconversion. For participants who were positive by PCR but who were negative for HIV antibody, the date of the positive PCR was used as the date of infection. In both groups, time from enrolment was accumulated up to the 24 month follow-up visit and HIV incidence was estimated per 100 person-years.

	Intervention group (n=2474)	Control group (n=2522)
Age (years)		
15–19	679 (27%)	719 (29%)
20–24	686 (28%)	686 (27%)
25–29	440 (18%)	473 (19%)
30–49	669 (27%)	643 (25%)
Marital status		
Never married	1161 (47%)	1222 (48%)
Currently married	1167 (47%)	1173 (47%)
Previously married	146 (6%)	127 (5%)
Religion		
Catholic	1649 (67%)	1730 (69%)
Protestant	667 (27%)	629 (25%)
Saved/Pentecostal/other	141 (6%)	146 (6%)
Muslim	17 (0·7%)	17 (0·7%)
Education		
No education	141 (6%)	147 (6%)
Primary	1631 (66%)	1669 (66%)
Secondary	603 (24%)	589 (23%)
Post-secondary	99 (4%)	116 (5%)
Number of sexual partners in the past year		
0	468 (19%)	494 (20%)
1	1152 (47%)	1168 (46%)
2	545 (22%)	586 (23%)
3+	309 (12%)	274 (11%)
Non-marital partners in the past year		
No	1220 (49%)	1238 (49%)
Yes	1254 (51%)	1284 (51%)
Condom use past year		
None	978 (40%)	941 (37%)
Inconsistent use	689 (28%)	732 (29%)
Consistent condom use	339 (14%)	355 (14%)
Alcohol use with sex in past 6 months	938 (38%)	966 (38%)
Transactional sexual intercourse*	38 (2%)	36 (1%)
Prior receipt of voluntary counselling and testing	648 (26%)	574 (23%)
Self-reported symptoms of sexually transmitted diseases in past year		
Genital ulcer disease	179 (7%)	176 (7%)
Urethral discharge	85 (3%)	94 (4%)
Dysuria	138 (6%)	162 (6%)

Data are n (%). Percentages have been rounded. *Sexual intercourse for money or gifts.

Table 2: **Enrolment characteristics, risk behaviours, and symptoms of sexually transmitted diseases by study group**

Exploratory analyses assessed the comparability of the two study groups at enrolment. HIV incidence during the trial was assessed by fixed covariates such as age, marital status, and education at enrolment, and by time-varying covariates such as sexual risk behaviours (eg, number of partners, non-marital relationships, condom use, and alcohol use), and symptoms of sexually transmitted diseases reported at follow-up visits. Men who were originally allocated to circumcision but who did not present for surgery within 6 months of enrolment were assessed as crossovers, as were individuals in the control group who opted to have circumcisions done outside the study.

We used a modified intention-to-treat approach for the primary efficacy analysis, which included all participants who were serologically or PCR negative at enrolment. Three participants who were PCR-positive but antibody negative at enrolment were deemed to have been infected before randomisation and were excluded from this modified intention-to-treat analysis. The primary modified intention-to-treat population included crossovers and participants who reported periods of sexual abstinence during the 24 months of follow-up. Incidence rate ratios (IRR) and 95% CI of HIV acquisition in the intervention versus the control group were estimated via exact methods, with Poisson multiple regression used for the adjusted analyses, including trend assessments. Because the trial was ended early, the Poisson analysis for the 0–24 month interval is weighted by the preponderance of person-time accrued during the first 12 months, and thus is a conservative estimate. Primary analyses adjusted for postulated potential confounders identified in previous studies in Rakai[8] and included baseline values of age, marital status, and sexual risk behaviours. Time varying covariates (eg, self-reported genital ulcer disease) could be in the causal pathway, so were not adjusted for during follow-up. We did an as-treated analysis that included control crossover participants who had received circumcision from outside sources, with person-time in the circumcised state ascribed to the beginning of the follow-up interval in which the surgery occurred. For crossovers in the intervention group who did not receive surgery, person-time was ascribed to the uncircumcised state from time of enrolment. Poisson multiple regression models were fit for the whole population and for strata of particular interest (eg, self-reported genital ulcer disease).

We did a Kaplan-Meier estimation based on analyses of time-to-detection of HIV infection at the visit at which positive serology or PCR was first identified. Due to the discrete nature of the timing of follow-up, data from visits were ascribed to the time of scheduled follow-up visits. An overall risk difference and risk ratios were calculated at the end of follow-up, with CI based on standard Greenwood formula variance estimates. The Kaplan-Meier risk ratios are not affected by the early trial closure, and this method was used in both other trials of male circumcision. Therefore, we present Kaplan-Meier risk ratios for comparative purposes.

To assess possible behavioural disinhibition, risk behaviours were tabulated by follow-up visit, and differences between study groups were assessed by χ^2 and Fisher exact tests. Symptoms of sexually transmitted diseases reported at each visit were cumulated over the 24 months of follow-up to estimate the prevalence of symptoms per 100 visits in intervention and control participants. Prevalence risk ratios (PRR) were estimated with log-binomial regression with a robust variance adjustment to account for within-person correlation. We also examined possible associations between reported symptoms of sexually transmitted diseases and incident HIV infection, by use of subgroup-specific models to determine whether any effects of circumcision on HIV incidence might be mediated by symptomatic sexually transmitted disease cofactors.

The frequencies of adverse events both related and unrelated to study participation were assessed in both study groups. Multiple adverse events diagnosed at a single visit were counted as separate events despite the fact that they could have been causally related (eg, wound dehiscence and infection), to provide an estimate of the maximum frequency of adverse events without making assumptions about causality.

The study had 80% power to detect a rate ratio of 0·5 for incident HIV in the intervention group relative to the control group, with a projected total person-time of 8993 person-years, assuming a 15% annual loss to follow-up and 10% crossover over 24 months. Formal statistical monitoring used the Lan-DeMets group sequential approach[9] with an O'Brien-Fleming type α spending function[10] to minimise the chance of inappropriate premature trial termination. Two interim analyses were done, the first with a data cutoff date of April 30, 2006, when about 43% of projected person-time had been accrued, and the second interim analysis with a data cutoff date of Oct 31, 2006, when about 72% of projected person-time had been accrued. The second interim analysis showed a significant difference in HIV incidence between the two study groups (nominal α=0·0215); as a result, NIAID terminated the trial for efficacy on Dec 12, 2006. The analyses presented here are based on all data accrued up to the time of trial closure in December, 2006, and encompass about 73% of total anticipated person-time. Results were deemed to be statistically significant at the α=0·05 level. All data were double entered. East was used for spending function calculations and Stata version 8 was used for analysis.

This trial is registered with ClinicalTrials.gov, with the number NCT00425984.

Role of the funding source

This trial was funded through a cooperative agreement with the Division of AIDS, NIAID/NIH. The study was done by the Rakai Health Sciences Program, a research collaboration between the Uganda Virus Research Institute, and researchers at Makerere University and Johns Hopkins University and Columbia University. FM, LHM, and MAC had full access to all the data until the trial closed. Thereafter, the principal investigator and co-investigators (RHG, GK, DS, MJW, FN, NKS, FWM, AND SJR) had access to all the data. Staff at the Division of AIDS maintained oversight of progress and reporting, and participated in study conduct and data interpretation as members of the study executive committee. Data analyses was done by the research teams at John Hopkins University and the Rakai Health Sciences Program. The corresponding author had final responsibility for preparing and submitting results for publication.

	Intervention group	Control group	Incidence rate ratio (95% CI)	p value
0–6 months follow-up interval				
Number of participants	2263	2319		
Incident events	14	19		
Person-years	1172·1	1206·7		
Incidence per 100 person-years	1·19	1·58	0·76 (0·35–1·60)	0·439
6–12 months follow-up interval				
Number of participants	2235	2229		
Incident events	5	14		
Person-years	1190·7	1176·3		
Incidence per 100 person-years	0·42	1·19	0·35 (0·10–1·04)	0·0389
12–24 months follow-up interval				
Number of participants	964	980		
Incident events	3	12		
Person-years	989·7	1008·7		
Incidence per 100 person-years	0·30	1·19	0·25 (0·05–0·94)	0·0233
Total 0–24 months follow-up				
Cumulative number of participants	2387	2430		
Cumulative incident events	22	45		
Cumulative person-years	3352·4	3391·8		
Cumulative incidence per 100 person-years	0·66	1·33	0·49 (0·28–0·84)	0·0057

Table 3: **HIV incidence by study group and follow-up interval, and cumulative HIV incidence over 2 years**

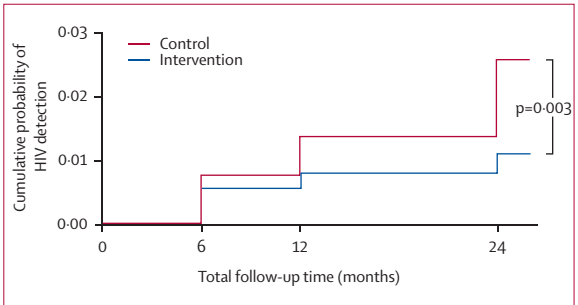

Figure 2: **Kaplan-Meier cumulative probabilities of HIV detection by study group**
Actual visits grouped by the three scheduled visits at 6 months, 12 months, and 24 months after enrolment. The cumulative probabilities of HIV infection were 1·1% in the intervention group and 2·6% in the control group over 24 months.

Results

Figure 1 shows the trial profile. 5000 eligible men were initially enrolled. However, during follow-up we discovered that four men (two in each study group) had re-enrolled under assumed names. For these individuals, the first enrolment record was retained in the dataset for the primary intent-to-treat analysis and the second enrolment was deleted, leaving 4996 enrolled participants. 146 (6%) participants in the intervention group did not come for surgery within 6 months of randomisation and

	Intervention group		Control group		Incidence rate ratio (95% CI)
	HIV incidence/ person-years	HIV incidence (cases per 100 person-years)	HIV incidence/ person-years	HIV incidence (cases per 100 person-years)	
Characteristics at enrolment					
Age (years)					
15–19	4/928·5	0·43	6/963·7	0·63	0·69 (0·14–2·92)
20–24	9/931·1	0·97	18/932·1	1·93	0·50 (0·20–1·17)
25–29	6/589·1	1·02	12/627·5	1·91	0·53 (0·16–1·53)
30–49	3/903·8	0·33	9/868·5	1·04	0·32 (0·06–1·28)
Marital status					
Never married	8/1575·5	0·51	18/1636·4	1·10	0·46 (0·17–1·12)
Currently married	10/1588·3	0·63	19/1582·4	1·20	0·52 (0·22–1·19)
Previously married	4/188·6	2·12	8/172·9	4·63	0·46 (0·10–1·71)
Education					
No education/primary	15/2385·3	0·63	32/2397·1	1·33	0·47 (0·24–0·90)
Secondary education	8/835·3	0·72	11/832	1·32	0·54 (0·16–1·60)
Post-secondary education	1/131·8	0·76	2/161·6	1·24	0·61 (0·01–11·78)
Behaviour and symptoms of sexually transmitted infections during follow-up					
Number of sexual partners					
0	3/590·3	0·51	3/661·8	0·45	1·12 (0·15–8·37)
1	14/1766·8	0·79	25/1720·3	1·45	0·55 (0·26–1·09)
2+	5/905·3	0·55	17/930·4	1·83	0·30 (0·09–0·85)
Type of relationship					
No non-marital relationships	15/2215·0	0·68	24/2251·9	1·07	0·64 (0·31–1·26)
Non-marital sexual partners	7/1047·5	0·67	21/1060·5	1·98	0·34 (0·12–0·82)
Condom use					
No condom use*	9/1233·1	0·73	14/1295·6	1·08	0·68 (0·29–1·56)
Inconsistent condom use*	7/939·4	0·75	21/885·7	2·37	0·31 (0·11–0·77)
Consistent condom use*	3/499·7	0·60	7/469·4	1·49	0·40 (0·07–1·76)
Alcohol use					
No alcohol use with sexual intercourse*	4/1315·7	0·30	14/1182·9	1·18	0·26 (0·06–0·82)
Alcohol use with sexual intercourse*	15/1356·5	1·11	28/1467·7	1·91	0·58 (0·29–1·12)
Transactional sexual intercourse					
No*	19/2633·9	0·72	41/2615·9	1·57	0·46 (0·25–0·81)
Yes*	0/37·7		1/34·7	2·88	0·00 (0·00–35·9)
Genital ulceration					
No genital ulcers	20/3153·1	0·63	33/3122·6	1·06	0·60 (0·33–1·08)
Genital ulcers	2/109·9	1·82	12/189·8	6·32	0·29 (0·03–1·29)
Urethral discharge					
No discharge	20/3198·3	0·63	39/3241·4	1·20	0·52 (0·28–0·91)
Urethral discharge	2/64·7	3·09	6/71·0	8·45	0·37 (0·04–2·05)
Dysuria					
No dysuria	20/3151·5	0·63	40/3203·0	1·25	0·51 (0·28–0·89)
Dysuria	2/111·5	1·79	5/109·4	4·57	0·39 (0·04–2·40)

*Among those sexually active in the follow-up interval.

Table 4: **Cumulative HIV incidence over 24 months by sociodemographic characteristics at enrolment, and behavioural characteristics and symptoms of sexually transmitted infections during follow-up**

	Intervention group		Control group		Prevalence risk ratio (95% CI)*	p value
	Episodes/number of visits	Rate (%)	Episodes/number of visits	Rate (%)		
Genital ulcer disease	168/5494	3·1%	322/5564	5·8%	0·53 (0·43–0·64)	<0·0001
Genital discharge	99/5494	1·8%	120/5564	2·2%	0·84 (0·63–1·11)	0·21
Dysuria	176/5494	3·2%	184/5564	3·3%	0·97 (0·77–1·21)	0·78

*Based on robust variance estimates adjusting for multiple observations on the same individuals

Table 5: **Prevalence of self-reported symptoms of sexually transmitted infections per visit, cumulatively over 24 months follow-up**

were classified as crossovers. Among the controls, 33 men were circumcised from other sources, a crossover rate of 1·3%. There were 15 deaths among participants in the intervention group over 3352·4 person-years and 17 deaths in the control group over 3391·8 person-years (4·5 deaths per 1000 person-years *vs* 5·0 deaths per 1000 person-years, p=0·8). None of the deaths were related to trial participation.

Trial retention rates are shown in table 1. All 1 year follow-up visits had been completed at time of trial termination, and retention rates at 12 months were equivalent in both groups. By December 12, 2006, the date of trial termination, 44% of men in both groups had reached their 24 month follow-up time point; retention rates for these men were much the same in both groups.

The baseline characteristics of the enrolled participants are shown in table 2. The two arms were much the same in terms of sociodemographic characteristics (age, marital status, religion, and education) and in sexual risk behaviours (number or partners, condom use, alcohol consumption with sex, and sex for money or gifts). At enrolment, previous receipt of voluntary counselling and testing was slightly higher in the intervention group than in the control group. The two groups reported similar rates of symptoms of sexually transmitted infections.

Table 3 shows HIV incidence by study arm and follow-up visit intervals, together with cumulative incidence over 2 years. The intention-to-treat analysis showed a progressive decrease in incidence in the intervention group over the entire follow-up period (p for trend 0·014). Incidence fell in the control group between the time of first follow-up and the time of second follow-up, and remained stable thereafter; however, the trend was not significant (p=0·6). The IRR of HIV acquisition associated with circumcision also fell over time; this increase in efficacy was of borderline significance (p=0·054 for the time-by-study arm interaction). The 24 month cumulative HIV incidence was 0·66 cases per 100 person-years in the intervention group, compared with 1·33 cases per 100 person-years in the control group. The unadjusted IRR was 0·49 (95% CI 0·28–0·84; p=0·0057). After adjustment for age, marital status, and sexual risk behaviours at enrolment, the IRR was 0·49 (0·29–0·81; p=0·003). Figure 2 shows the Kaplan-Meier survival curves for time-to-detection of HIV infection for the modified intention-to-treat analysis. The difference between the cumulative probabilities of HIV detection was significant (p=0·003) and the risk ratio was 0·43 (0·24–0·75). The as-treated Poisson analysis, which assigned person-time according to the actual circumcision status of participants, showed an incidence of 0·61 cases per 100 person-years in the intervention group (20 events in 3268·1 person-years), and 1·35 cases per 100 person-years in the control group (47 events in 3481·6 person-years) with an IRR of 0·45 (95% CI 0·25–0·78; p=0·0022). The as-treated Kaplan-Meier risk ratio was 0·40 (0·23–0·70, p=0·003).

Table 4 shows cumulative HIV incidence over 24 months by sociodemographic characteristics at enrolment, and by self-reported sexual risk behaviours and symptoms of sexually transmitted infections during follow-up. The rates of HIV acquisition were lower among circumcised men in all strata of characteristics, risk behaviours and symptoms of sexually transmitted infections examined, with the exception of those men who reported no sexual activity within the follow-up interval of seroconversion. HIV incidence was highest in the 25–29 year age-group, but in all age-groups, incidence was lower in the intervention than in the control group. Similarly, HIV incidence was lower in circumcised than in uncircumcised men in all categories of marital status and education. Among sexually active men, circumcision reduced HIV acquisition irrespective of the number of partners, non-marital relationships, condom use, consumption of alcohol before sexual intercourse, and transactional sexual intercourse. Men reporting symptoms of sexually transmitted diseases during a follow-up interval had higher rates of HIV acquisition than did asymptomatic participants, but the protective effects of circumcision were observed irrespective of the presence of such symptoms. However, circumcision was not protective against HIV acquisition in the few men who reported no sexual activity in a given follow-up interval. There were six incident cases (three in each group) during periods of reported abstinence. None of these six participants reported receipt of injections or transfusions during the follow-up interval of HIV seroconversion; these participants probably under-reported their sexual activity.

The prevalence rates of self-reported symptoms of sexually transmitted diseases reported at each follow-up visit, cumulated over 24 months, are shown in table 5. Over all study visits, the prevalence of self-reported genital ulcers during the preceding interval was lower in the

	Intervention group	Control group	p value
6 months follow-up (reference period 6 months since enrolment)			
Total number seen	2268 (100%)	2321 (100%)	
Number of sexual partners			0·1
0	467 (21%)	534 (23%)	
1	1263 (56%)	1223 (53%)	
2	407 (18%)	435 (19%)	
3+	131 (6%)	129 (6%)	
Non-marital partners*	697 (39%)	704 (39%)	0·8
Consistent condom use*	334 (19%)	295 (17%)	0·11
Inconsistent use*	662 (37%)	557 (31%)	0·0004
No condom use*	805 (45%)	935 (52%)	<0·0001
Alcohol use with sexual intercourse*	889 (49%)	981 (55%)	0·001
Transactional sexual intercourse*	29 (2%)	29 (2%)	1·0
12 months follow-up (reference period 6 months)			
Total number seen	2253 (100%)	2250 (100%)	
Number of sexual partners			0·4
0	437 (19%)	477 (21%)	
1	1249 (56%)	1201 (53%)	
2	463 (21%)	458 (20%)	
3+	103 (5%)	114 (5%)	
Non-marital partners*	699 (39%)	692 (39%)	0·9
Consistent condom use*	333 (18%)	323 (18%)	0·9
Inconsistent use*	533 (29%)	536 (30%)	0·6
No condom use*	949 (52%)	914 (52%)	0·7
Alcohol use with sexual intercourse*	962 (53%)	996 (56%)	0·06
Transactional sexual intercourse*	21 (1%)	17 (1%)	0·6
24 months follow up (reference period 12 months)			
Total number seen	978 (100%)	995 (100%)	
Number of sexual partners			0·8
0	131 (13%)	145 (15%)	
1	499 (51%)	498 (50%)	
2	247 (25%)	244 (25%)	
3+	100 (10%)	108 (11%)	
Non-marital partners*	335 (40%)	350 (41%)	0·7
Consistent condom use*	158 (19%)	160 (19%)	1·0
Inconsistent use*	332 (39%)	331 (39%)	0·9
No condom use*	356 (42%)	359 (42%)	0·9
Alcohol use with sexual intercourse*	429 (51%)	481 (57%)	0·02
Transactional sexual intercourse*	11 (1%)	12 (1%)	0·8

Date are n (%). *Among those who reported sexual activity in the follow-up interval.

Table 6: **Sexual risk behaviours by study group and follow-up visit**

intervention group than in the control group (3·1% *vs* 5·8%; PRR 0·53, 95% CI 0·43–0·64; p<0·0001). However, circumcision had little effect on the prevalence of urethral discharge or dysuria.

To assess possible behavioural disinhibition, sexual risk behaviours were assessed at each follow-up visit (table 6). During the first 6 month follow-up interval, sexual activity was reported by 1801 (79%) participants in the intervention group, compared with 1787 (77%) of those in the control group (p=0·049). Consistent condom use during this interval was slightly higher in the intervention group than it was in the control group (table 6; p=0·11). Similarly, inconsistent condom use was higher in the intervention group than it was in the control group (table 6; p=0·0004). At the 12 and 24 months follow-up visits, the number of sexual partners, non-marital relationships, and condom use were much the same in the two groups. However, participants in the control group reported slightly higher rates of alcohol use with sexual intercourse in all follow-up intervals than did those in the intervention group; this was significant at the 6 month (p=0·001) and 24 month (p=0·02) visits (table 6). Transactional sexual intercourse was infrequent and did not differ between study groups. There is, therefore, no consistent or substantial evidence of behavioural disinhibition after circumcision in the study population.

Adverse events unrelated to trial participation were frequent. 1391 adverse events were reported in the intervention group, compared with 1320 in the control group (56% *vs* 52%; p=0·083). Of these adverse events, 1213 (87%) in the intervention group were unrelated to the trial; all adverse events in the control group were unrelated to the trial. Almost half of the unrelated adverse events were mild grade 1 events (46% [n=558] of those in the intervention group and 50% [n=660] of those in the control group). The rate of all adverse events related to surgery in the intervention group was about 8% (178 events in 2328 surgeries); most of these events were mild (94 of 178 events). The rate of moderate adverse events related to surgery was about 3% (79 events in 2328 surgeries), and there were five severe adverse events, with a rate of 0·2 events per 100 surgeries. The severe adverse events included one wound infection, two haematomas that required re-exploration and ligation of active bleeding vessels, one wound disruption due to external cause, and one case of severe postoperative herpetic ulceration not involving the surgical wound requiring hospitalisation in the programme's facility. All moderate and severe adverse events were successfully managed and resolved.

Discussion

This large, randomised trial of adult male circumcision in a rural Ugandan population shows that such a surgical intervention reduces the risk of the acquisition of HIV in men. We noted a significant reduction in HIV incidence among circumcised men compared with uncircumcised control participants. The efficacy of circumcision for prevention of incident HIV was 51% in the Poisson intention-to-treat analysis; adjustment for enrolment characteristics, behaviours, and symptoms of sexually transmitted infections did not affect this estimate. In the as-treated Poisson analysis, efficacy was 55% and the Kaplan-Meier estimate of efficacy was 60%. These findings are compatible with observational data,[1–5] as well as data from a randomised trial in South Africa (60% intention-to-treat efficacy and 76% as-treated efficacy in a semi-urban population aged 18–24 years),[6] and a trial in Kenya (53% intention-to-treat efficacy

and 60% as-treated efficacy in an urban population, aged 18–24 years),[11] suggesting similar efficacy in widely divergent populations. Thus, circumcision must now be deemed to be a proven intervention for reducing the risk of heterosexually acquired HIV infection in adult men.

HIV incidence in the intervention group fell significantly over time, whereas it remained fairly constant in the control group, and the protective efficacy of circumcision increased progressively during later follow-up intervals (eg, 75% efficacy during the 12–24 month follow-up interval, table 3). The Kaplan-Meier curves for time to detection of HIV infection did not diverge until the twelfth month of follow-up, meaning that the difference in HIV acquisition began during the 6–12 month follow-up interval (figure 2). The HIV incidence in the control group (1·3 cases per 100 person-years), is identical to that seen in uncircumcised men in the Rakai population at the time the trial was done.[12] Also, 45% of HIV-negative uncircumcised men in the Rakai cohort volunteered to enroll in the trial, which suggests that the trial results are probably generalisable to the Rakai population as a whole. At the time of trial closure, 80% of eligible control participants who had completed 24 months follow-up agreed to be circumcised, suggesting high acceptability.

We did not find evidence that men in the intervention group adopted higher sexual risk behaviours than did those in the control group (table 6). This could have been due to the intensive health education provided during the trial to minimise risk compensation. These findings differ from those from the South African trial, which reported an increase in the mean number of sexual contacts in men in the intervention group.[6] Future circumcision programmes must emphasise that circumcision provides only part protection, and that there is a critical need to practise safer sex after circumcision (eg, partner limitation and consistent condom use).

Circumcision also reduced the rate of self-reported symptoms of genital ulcer disease with a cumulative efficacy of 48% over all follow-up visits (table 5), which is comparable with the protective effects of circumcision on genital ulcer disease in observational studies.[13] At this time, we cannot determine whether the procedure reduced the incidence of ulcerative infections due to syphilis, herpes simplex virus 2, and *Haemophilus ducreyi*, or whether removal of the prepuce reduced the severity, duration, or recurrence of ulceration, leading to lower recognition of symptoms. Since genital ulcer disease is a risk factor for the acquisition of HIV,[14–16] and symptomatic genital ulcer disease was associated with higher rates of HIV acquisition in this trial (table 4), it is plausible that the protective effect of circumcision on HIV could be mediated in part by the protective effects of the procedure on self-reported genital ulcer disease. By contrast, there was no effect of circumcision on symptoms of discharge or dysuria (table 5), which is consistent with data from observational studies that indicate a lack of an effect of circumcision on gonorrhoea or chlamydia prevalence.[3,17] The finding is biologically plausible since it suggests that circumcision could be protective against cutaneously acquired infections harboured in the moist subpreputial space, but the procedure does not seem to be protective against urethral infections, which presumably are unaffected by the removal of the foreskin.

That circumcision reduces the risk of male HIV infection is biologically plausible. The foreskin is rich in HIV target cells (Langerhans' and dendritic cells, CD4+ T cells, and macrophages),[18–21] and the inner preputial mucosa is unkeratinised, making it vulnerable to HIV infection.[20,22] The foreskin is retracted over the shaft during intercourse, which exposes the inner mucosa to vaginal and cervical fluids.[22] Also, breaches in the mucosa can occur due to microtears during intercourse, especially at the frenulum,[22] and uncircumcised men are more susceptible to genital ulcer disease, which could increase HIV entry.[13,22]

The 24 month transmission risks were 2·6% in the control group and 1·11% in the intervention group, giving a risk difference of 1·49%. Thus, assuming completion of 24 months of follow-up, we estimate that about 67 circumcisions are needed to prevent one HIV infection in the 2-year postoperative interval. However, this estimate does not include possible reductions in secondary transmissions to women or the probable long-term effectiveness of circumcision in men. Mathematical models have been used to estimate the number of surgeries required per HIV infection averted in both men and women over varying periods of time. In Rakai, a stochastic simulation model suggested that, with a circumcision efficacy of 50% and an HIV incidence of 1·3 per 100 person-years in uncircumcised men, the number of surgeries per HIV infection averted over 10 years was about 35, assuming all uncircumcised men accept the procedure.[12] In South Africa, with a circumcision efficacy of 60% and HIV incidence among uncircumcised men of 3·8 per 100 person-years, the number of surgeries per infection averted over 20 years is much lower.[23] Thus, the number of surgeries needed to prevent one HIV infection will vary depending on background HIV incidence, the level of acceptance, and the duration of projected protection. Policymakers will have to determine whether adult male circumcision is likely to be an appropriate and cost-effective intervention in specific settings. In the longer term, neonatal circumcision or circumcision of younger boys will provide a simpler, safer, and cheaper option, although the HIV benefits will be delayed until these boys reach sexual maturity.

Adult male circumcision is not without risk. In this trial the rate of moderate and severe adverse events related to surgery was almost 4%, which is comparable with rates in the South African and Kenyan trials.[6,9] One should note that there were cases in which appropriate follow-up management was required to prevent more serious sequelae. Furthermore, substantially higher complication rates have been reported when surgery is done in rural clinics or by traditional circumcisers.[24] The scale-up of

circumcision services will require careful attention to training of personnel, provision of facilities, equipment and supplies, postoperative care to minimise and manage complications, and monitoring of the quality of services and surgical outcomes.

The use of surgery for disease prevention is an unusual public-health intervention. One precedent is the mass sterilisation camps in India during the 1970s, which were poorly implemented and resulted in serious surgical complications, deaths, and ultimately the collapse of the programmes.[25,26] Thus, future provision of circumcision for HIV prevention must maintain the highest achievable levels of safety to be acceptable and sustainable.

The consistency of epidemiological evidence from three randomised trials and multiple observational studies presents a compelling case for the promotion of male circumcision for HIV prevention in populations where circumcision is infrequently practiced and where HIV transmission is mainly due to heterosexual intercourse. Such practice is especially relevant in east and southern Africa, where circumcision rates are low in many populations and the HIV epidemic is most severe. However, trials that are stopped early could overestimate efficacy when compared with subsequent studies[27] and to undertake long-term post-circumcision trial surveillance is essential to determine the effectiveness of circumcision in populations with varying HIV prevalence, and to assess the durability of any observed benefits. Furthermore, to assess whether perceptions of circumcision efficacy lead to an exaggerated belief in the protective effects of the procedure, thus engendering increases in HIV risk behaviours, will be important.

Contributors

All authors took part in the design, implementation, and analysis of this study and saw and approved the final version.

Conflict of interest statement

We declare that we have no conflict of interest.

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Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial

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Summary

Background **Male circumcision could provide substantial protection against acquisition of HIV-1 infection. Our aim was to determine whether male circumcision had a protective effect against HIV infection, and to assess safety and changes in sexual behaviour related to this intervention.**

Methods **We did a randomised controlled trial of 2784 men aged 18–24 years in Kisumu, Kenya. Men were randomly assigned to an intervention group (circumcision; n=1391) or a control group (delayed circumcision, 1393), and assessed by HIV testing, medical examinations, and behavioural interviews during follow-ups at 1, 3, 6, 12, 18, and 24 months. HIV seroincidence was estimated in an intention-to-treat analysis. This trial is registered with ClinicalTrials.gov, with the number NCT00059371.**

Findings **The trial was stopped early on December 12, 2006, after a third interim analysis reviewed by the data and safety monitoring board. The median length of follow-up was 24 months. Follow-up for HIV status was incomplete for 240 (8·6%) participants. 22 men in the intervention group and 47 in the control group had tested positive for HIV when the study was stopped. The 2-year HIV incidence was 2·1% (95% CI 1·2–3·0) in the circumcision group and 4·2% (3·0–5·4) in the control group (p=0·0065); the relative risk of HIV infection in circumcised men was 0·47 (0·28–0·78), which corresponds to a reduction in the risk of acquiring an HIV infection of 53% (22–72). Adjusting for non-adherence to treatment and excluding four men found to be seropositive at enrolment, the protective effect of circumcision was 60% (32–77). Adverse events related to the intervention (21 events in 1·5% of those circumcised) resolved quickly. No behavioural risk compensation after circumcision was observed.**

Interpretation **Male circumcision significantly reduces the risk of HIV acquisition in young men in Africa. Where appropriate, voluntary, safe, and affordable circumcision services should be integrated with other HIV preventive interventions and provided as expeditiously as possible.**

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Introduction

Although the availability of antiretroviral therapy for individuals infected with HIV is increasing worldwide, many more new infections are occurring for every additional person started on such treatment.[1] Prevention of new infections is the only realistic hope for stemming the HIV pandemic, yet currently available prevention measures have often been unsuccessful in restricting the spread of HIV, and there is little promise that an effective vaccine will be available within the next 15 years.[2] Effective new HIV preventive interventions are needed.

That male circumcision might reduce risk of HIV acquisition was first proposed in 1986.[3,4] Ecological studies have shown that, in regions where HIV transmission is predominantly heterosexual, the prevalence of HIV and of male circumcision are inversely correlated.[5–8] More than 30 cross-sectional studies have found the prevalence of HIV to be significantly higher in uncircumcised men than in those who are circumcised,[9] and 14 prospective studies all show a protective effect, ranging from 48% to 88%.[9–13] A systematic review and meta-analysis of studies from sub-Saharan Africa reported an adjusted relative risk of 0·42 (95% CI 0·34–0·54) in all circumcised men, with a stronger adjusted relative risk of 0·29 (0·20–0·41) in circumcised men who were at higher risk of acquiring HIV.[14] In a cohort study of Ugandan discordant couples in which the female was HIV infected and the male partner was initially HIV seronegative, 37 of 134 uncircumcised men versus none of 50 circumcised men became seropositive after about 2 years of follow-up.[15]

Biological studies suggest a plausible mechanism for this protection. The inner mucosal surface of the human foreskin, exposed upon erection, has nine times higher density of HIV target cells (Langerhans' cells, CD4+ T cells, and macrophages) than does cervical tissue.[16] The number of preputial target cells is increased in men with a history of recent sexually transmitted infections.[17] By contrast with the foreskin's inner surface, HIV target cells on the outer surface and the glans are protected by a layer of squamous epithelial cells.[16,18] In explant culture, several times more HIV-1 is taken up by Langerhans' cells and CD4+ T cells in foreskin than in cervical tissue; the virus does not infiltrate cells on the outer surface of the foreskin.[16] Other possible mechanisms by which the presence of the foreskin could lead to greater risk for HIV infection include poor hygiene,[19] greater incidence of ulcerative sexually transmitted infections,[20] and susceptibility of the foreskin to abrasions.[9]

Recently, a randomised controlled trial of male circumcision in 18–24-year-old men in Orange Farm,

South Africa, was stopped by the data and safety monitoring board when an interim analysis showed a 60% protective effect of circumcision in an intention-to-treat analysis, and a 76% protective effect in a per-protocol analysis that adjusted for crossovers. There were 20 HIV infections (incidence rate 0·85 per 100 person-years) in the circumcision group and 49 (2·1 per 100 person-years) in the uncircumcised group. Controlling for behavioural factors—eg, condom use, health-seeking behaviour, and sexual behaviour—the protective effect was much the same (61%).[21]

Upon announcement of the Orange Farm results in July, 2005,[22] the WHO and UN agencies issued a statement indicating that the evidence available up to that time for male circumcision having a protective effect against HIV infection was very promising, but that circumcision should not be promoted as a prevention strategy until results from this study, and a third trial in Rakai, Uganda, became available.[23] A Cochrane review had also cautioned against implementation of male circumcision as a preventive strategy in the absence of more data from clinical trials.[24]

Here we report the results of a randomised controlled trial of male circumcision in 18–24-year-old men in Kisumu, Kenya. Our aim was to determine the relative risk of HIV incidence in men randomly assigned to receive circumcision versus those who did not receive such treatment.

Methods

Participants

This trial was done in Kisumu district, Kenya. Kisumu is the capital city of Nyanza Province in western Kenya and has a population of about 500 000 residents.[25] Most residents self-identify as Luo, an ethnic group that does not traditionally practice circumcision. About 10% of Luo adult men in Kisumu are circumcised.[26] In 2003, HIV prevalence was about 25% in Luo women and 18% in Luo men.[27]

Participants were recruited via local newspapers, radio, fliers, and street shows by drama and musical groups. Recruitment began on Feb 4, 2002, and enrolment was completed on Sept 6, 2005. Public and private clinics were enlisted to refer patients with sexually transmitted infections, and peer outreach workers recruited participants from local youth organisations. Enrolled participants were each given up to three coupons valued at US$1·25 for every peer they recruited for initial screening. Potential participants were initially asked their residence, willingness to be tested for HIV, and proof of age. They were then seen privately by trained counsellors for HIV testing and counselling, verification of circumcision status, haemoglobin concentration, whether they were sexually active in the previous 12 months, and intention to remain in the area for at least 2 years. HIV-seropositive men were referred to a post-test counselling and support group established and supported by the project. Those individuals who were eligible were further informed about the trial, given a comprehensive consent form to read and study in any of three languages (English, Dholuo, and Kiswahili), and asked to return 2 days or more later. At the second screening visit, counsellors went through the consent form in detail. Participants who provided written informed consent had a medical examination, and a questionnaire was administered to assess sexual risk behaviours; blood was drawn and urine was collected for laboratory tests and repository; and urethral or penile swabs were taken if urethral discharge or genital ulcers were present. Participants with sexually transmitted infections or other treatable medical conditions were deferred until treated. Inclusion and exclusion criteria are listed in the panel. Participants were offered 300 Kenyan shillings (about $4) for each scheduled study visit to cover travel expenses and loss of income.

The research protocol was reviewed and approved by the Kenyatta National Hospital ethics and research committee, the University of Illinois institutional review board number three, the University of Manitoba biomedical research ethics board, the Research Triangle Institute institutional review board number one, and the University of Washington institutional review board. An advisory board of Kisumu community members from diverse backgrounds met about four times a year to advise the research team on conduct of the trial. The National Institute of Allergy and Infectious Diseases (NIAID) contracted WESTAT (Rockville, MD, USA) as the clinical site monitor for the trial. Monitoring visits occurred about three times per year. The NIAID vaccine and prevention data and safety monitoring board initially reviewed the protocol; periodically reviewed enrolment, data quality, adverse events, protocol deviations, and outcome measures; and gave advice based on results of interim analyses.

***Panel:* inclusion and exclusion criteria**

Inclusion criteria
Uncircumcised
HIV negative
Sexually active
Aged 18–24 years
Resident of Kisumu district
No plans to move for at least 2 years
Consent to participate
Haemoglobin 90 g/L or more

Exclusion criteria
Foreskin covers less than half the glans
Haemophiliac or other bleeding disorder
High prothrombin time index
Other medical condition contraindicating surgery
Absolute indication for circumcision

Procedures

Participants who met the study criteria were randomly assigned to either the intervention (circumcision) group or the control (delayed circumcision) group after being questioned to ensure their understanding of all study procedures and requirements for participation. Randomly permuted blocks of size 10 and 20 within age-groups of 18–20 years and 21–24 years were used to ensure approximately equal sample sizes in the two study groups within age strata. An opaque envelope system was used. The age stratum, the envelope number, and a randomisation identification number were printed on the outside of all envelopes. When a participant was ready for randomisation, the next envelope (based on envelope number) for the participant's age stratum was selected and the study coordinator wrote the participant's identification number on the outside of the envelope. The envelope was then opened by the participant and he read the assignment—circumcision or control—himself, in the presence of the study coordinator and one other staff member. The data coordinating centre routinely checked randomisation reports to validate compliance with the procedure. Men assigned to the circumcision group were scheduled for surgery the same day or shortly thereafter. Those assigned to the control group were asked to remain uncircumcised until the end of their 24 months of study participation, at which time they were offered circumcision at the study clinic.

All surgeries were done under local anaesthesia in the study clinic by study clinicians, using the standardised forceps-guided method described by Krieger and colleagues.[28] Participants were given verbal and written instructions on postoperative wound care, and were encouraged to come to the clinic or contact a study clinician at any time with medical problems. Postcircumcision visits were scheduled for 3, 8, and 30 days to check the wound, record any complications, and ask about sexual activity, level of pain, resumption of normal activities, and satisfaction with the procedure. Participants were counselled to refrain from sexual activity for at least 30 days after the procedure. Adverse events were assessed at every visit and classified as not related or possibly, probably, or definitely related to the surgical procedure. Severity was recorded as mild, moderate, or severe. All adverse events deemed to be possibly, probably, or definitely related to surgery were reviewed by more than one clinician. Regular case reviews were done with a local surgeon and the consultant urologist (JNK).

At each study visit—1, 3, 6, 12, 18 and 24 months after randomisation—all participants received HIV counselling and testing, underwent a genital examination to check circumcision status, and were asked questions about sexual activity. Follow-up was defined as incomplete with respect to HIV status if the participant had not been followed to seroconversion and a follow-up visit had been missed. Visits were deemed to be missed if 6 weeks late for the 1 month visit, 2 months late for the 3 month visit, or 5 months late for the 6, 12, 18, or 24 month visits.

At months 6, 12, 18, and 24, blood and urine were collected for diagnostic testing for sexually transmitted infections and repository, and an extensive questionnaire was administered to assess sexual function and behavioural factors associated with HIV infection. The nurse-counsellors who did the HIV testing and administered the questionnaire were blinded to study group, unless the participant divulged his circumcision status during counselling. All participants were provided free medical treatment throughout their 24 months of follow-up. Individually tailored risk reduction counselling occurred at every visit. Men who tested positive for a sexually transmitted infection were treated, received additional counselling, and were given a coupon for their sexual partner to receive free treatment at a neighbouring public clinic. Incident HIV-positive men were referred to the project's post-test counselling and support group and provided access to free HIV treatment and care. Condoms were provided free of charge to all men and their partners.

HIV serostatus and timing of seroconversion were determined as follows. If a participant was double positive or discordant on two rapid tests with the synthetic peptide test Determine HIV 1/2 (Abbott Diagnostic Division, Hoofddorp, Netherlands) and the recombinant antigen test Unigold Recombigen HIV Test (Trinity Biotech, Wicklow, Ireland) taken from the same fingerprick sample, then serum was drawn and sent to the International STD/HIV Collaborative Group laboratory at the University of Nairobi for double ELISA (Detect HIV 1/2, Adaltis Inc, Montreal, Canada, and Recombigen HIV 1/2, Trinity Biotech, Wicklow, Ireland). Results were available within 1 week. Participants were deemed to be confirmed positive if the ELISA tests were both positive. Two negative ELISA tests were considered negative; discordant ELISA tests were considered indeterminate and the participant was asked to return for additional testing 1–6 months later, depending on the visit. For purposes of determining serostatus for analysis of study data, blood specimens from all participants who tested positive on at least one rapid test and one ELISA test were sent to the Health Canada National HIV Reference Laboratory (Ottawa, Canada) for confirmatory testing by line immunoassay (INNO-LIA HIV 1/2, Immunogenetics NV, Ghent, Belgium). Specimens indeterminate by line immunoassay were tested by PCR at Health Canada or the Fred Hutchinson Cancer Research Center (Seattle, WA, USA), with the PCR result deemed to be definitive. Any participant confirmed as positive at a follow-up visit had his baseline specimen tested at the Health Canada laboratory to ascertain HIV serostatus at enrolment. Participants who had a confirmed positive test at the month 3 follow-up visit had their month 1 specimen tested by PCR. The HIV seroconversion visit was judged to be the first visit at which the participant had at least

one positive HIV rapid test and was confirmed as being HIV positive at the same or a subsequent visit according to the above procedure.

Statistical analysis

A target sample size of 2776 (1388 in each group) was set to detect a 50% difference in 2-year HIV seroincidence between the treatment groups, assuming a 15% non-informative loss-to-follow-up, 5% non-adherence to treatment assignment in either direction, 2·5 per 100 person-years annual HIV seroincidence in the control group, overall type I error rate of α=0·05 (two-sided), and 80% power. Two interim analyses and a final analysis were planned. Three interim analyses were done. The first used data accumulated through April 17, 2005, with about 37% of the potential follow-up experience accrued. This first analysis was assessed at α_1=0·000518 with the O'Brien and Fleming bound. The second analysis used data through May 13, 2006, with about 74% of the follow-up experience. The Lan and DeMets[29] spending function that preserves the O'Brien and Fleming bound while accounting more directly for the follow-up was used, and the bound for this second look at the data was α_2=0·0183. A third, unscheduled analysis was done at the request of the data and safety monitoring board using data through October 31, 2006, with about 87% of the follow-up experience accrued. By use of the same Lan and DeMets spending function, the stopping boundary for this third interim analysis was α_3=0·0269, and this boundary was crossed. On the recommendation of the data and safety monitoring board, the trial was stopped by the sponsor on December 12, 2006.

Data were recorded on paper forms and were then entered into a database at the study site via a customised data management system developed by the data coordinating at RTI International that included: data editing during data entry; tracking protocol visits and required forms; automated back-up and transmission processes; and system and database access security. Data were transmitted via the internet every night to the data coordinating centre. The coordinating centre did additional longitudinal data checks and posted queries on a study website for the clinic staff in Kisumu to review and to make corrections as appropriate. About 5% of study forms were re-keyed per month for quality assurance. The error rate at the item level was 0·3%.

The Kaplan-Meier[30] method was used to estimate the HIV event distribution over time by treatment, accounting for staggered enrolment and incomplete, discrete follow-up. The time of HIV-positive status was credited to the follow-up visit when HIV was first detected. HIV-negative participants were censored in the analysis at the last regular follow-up visit completed where HIV status was ascertained. Estimates of 2-year HIV seroincidences and corresponding standard errors obtained by Greenwood's formula[31] were used to test for differences between the treatments on the primary outcome (HIV seroconversion). The primary analysis was by intention-to-treat; participants were included in the analysis in the group to which they were randomly assigned and all participants with follow-up for HIV status were included in the analysis.

A secondary analysis, that used the same statistical approach described above, excluded participants subsequently confirmed as HIV positive by PCR at baseline, and one further analysis excluded those confirmed positive at either baseline or at 1 month. Furthermore, an as-treated analysis was done with a time-dependent covariate in a Cox regression model[32,33] for circumcision status at each follow-up visit to take into account those individuals who did not adhere to their randomisation assignment; in this analysis, a time-dependent variable for the circumcision status of each participant at each follow-up visit was constructed and included as a single time-dependent predictor variable in a Cox regression model with all participants. Thus, irrespective of treatment assignment, participants were accounted in this analysis as they were treated with respect to circumcision. Cox regression models with fixed covariates were used to consider various baseline adjustments to the treatment effect. Age-group and variables that seemed to be slightly imbalanced were used—ie, ethnic group, occupation, infection with herpes simplex virus type 2, and infection with *Chlamydia trachomatis*. These variables were considered independently for association with HIV incidence, then singly, as adjustments to the treatment effect. Finally, the set of variables was included in a model as an adjustment to the treatment effect.

All hazard or risk ratios were estimated with the parameter estimates from Cox regression. An exact method for computing the likelihood was specified to handle ties.

Behavioural outcomes were assessed in longitudinal analyses with the generalised estimating equations extension of generalised linear models proposed by Liang and Zeger.[34] Outcomes are binary, and for each specific outcome, the logit was modelled as a linear function of treatment, visit (month 0, 6, 12, 18, and 24) and the interaction of treatment and visit. The baseline response was included in the longitudinal stream. Visit was treated as a categorical variable and follow-up visits were compared with baseline. The interaction terms tested differences between treatment groups in change from baseline. Testing included an overall test of difference by treatment in the changes from baseline (four degrees of freedom test: month 6, 12, 18, and 24), and a test for difference by treatment in the specific change from baseline to month 24 (one degree of freedom test). No adjustment was made for multiple tests. The p values reported are those associated with Wald statistics, with empirical standard errors. The working correlation between measurements at any two follow-up times was specified as constant.

In addition to the methods used for the primary outcome and the behavioural outcome measures, the significance of

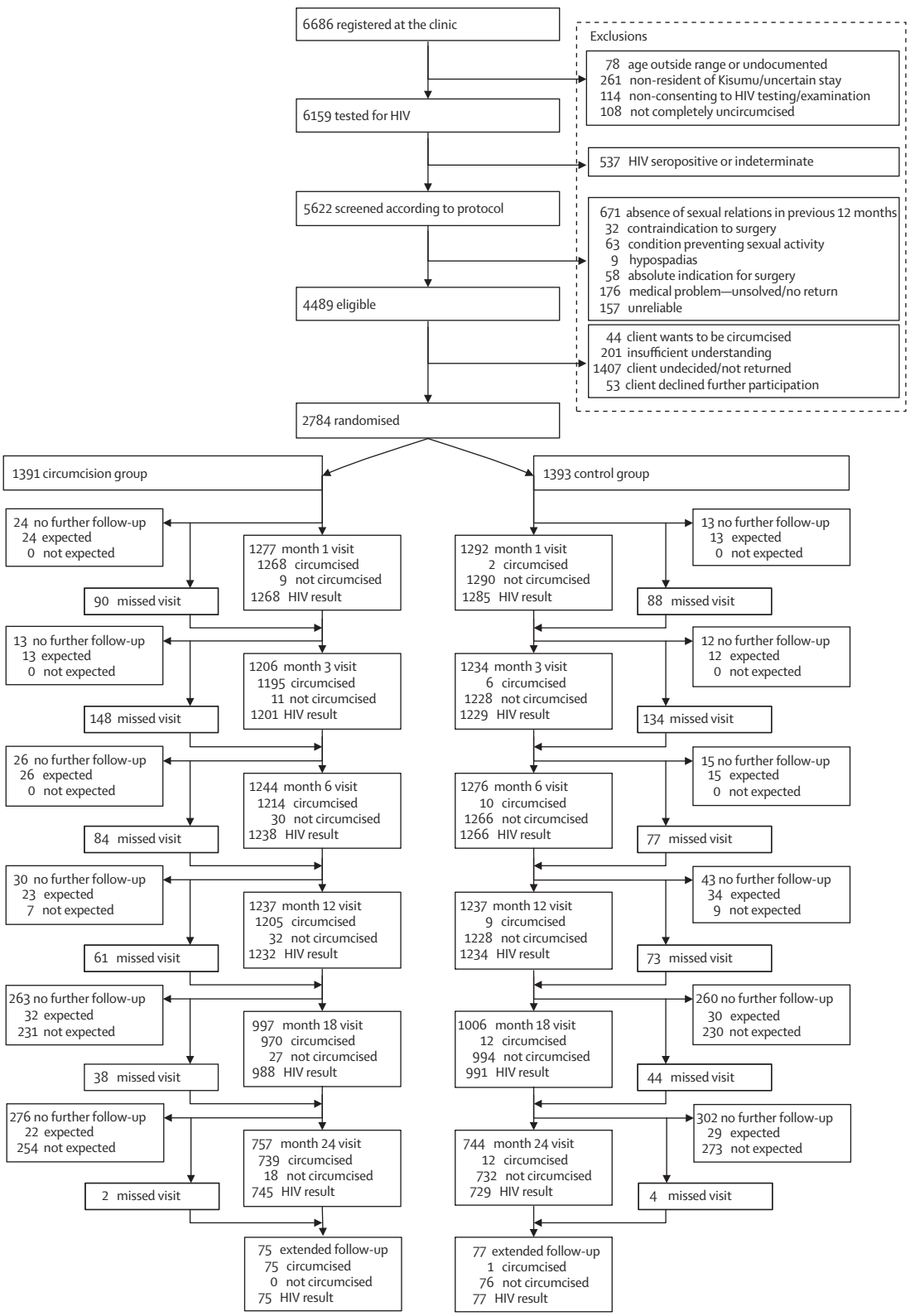

Figure 1: **Trial profile**
Because the exclusion categories were not mutually exclusive, exclusions might add up to more than the total number of individuals excluded. For each follow-up visit, participants with no further follow-up were classified as "expected" if they were eligible for that study visit but passed the window period and did not return for a subsequent visit. Those classified as "not expected" are those whose participation was truncated due to closure of the database on Oct 31, 2006. From March, 2006, participants who remained on study were invited to participate in an extended follow-up, beginning with 30 month visits in August, 2006. Numbers with extended follow-up visits are shown. Data from these visits could contribute outcome information (eg, negative status for HIV) accountable to previous visits for which no HIV test was available.

differences between groups was assessed with Fisher exact tests or χ^2 tests for proportions, Wilcoxon-Mann-Whitney tests for continuous and ordinal distributions, and log-rank tests for time-to-event distributions. All analyses are based on data available through Oct 31, 2006. All p values reported are two-sided. Analyses were done with SAS versions 8.2 and 9.1.

This trial is registered with ClinicalTrials.gov, with the number NCT00059371.

Role of the funding source

This trial was funded through a cooperative agreement with the Division of AIDS, NIAID/NIH and a grant from the Canadian Institutes for Health Research. The NIAID prevention and science review committee required minor revisions to the protocol. Only C B Parker had full access to all the data until the trial closed. Thereafter, the principal investigator and all co-investigators had access to all the data. Staff at the Division of AIDS maintained oversight of progress and reporting, and participated in study conduct and data interpretation as members of the study executive committee. RC Bailey had final responsibility for the decision to submit for publication.

Results

Figure 1 shows the trial profile. 6686 men initially came to the study clinic; 6159 (92%) met preliminary criteria. Of these, 478 (8%) were HIV seropositive, 59 (1%) were of indeterminate HIV status, and 5622 (91%) were seronegative. Of the seronegative individuals, 1133 (20%) were excluded for other reasons. Thus 4489 individuals were eligible for randomisation. Of these, 1407 were undecided or did not return for randomisation, 53 declined further participation, 201 were considered to have insufficient understanding of the protocol, and 44 wanted to be assigned to the circumcision group only. Thus, 2784 men were randomised: 1391 to the treatment (circumcision) group and 1393 to the control group.

The median age of the 2784 randomised participants was 20·0 years (IQR 19–22); of these individuals, 2739 (98%) identified themselves as Luo (table 1). Two-thirds (n=1837) had greater than a primary education and 1793 (64%) were unemployed. Most men identified themselves as unskilled workers, farm labourers, or fishermen (n=1653, 59%); 632 (23%) were students. Only about 7% reported being married or living with a partner. The treatment groups were much the same at baseline in terms of demographic characteristics, physical characteristics, prevalence of sexually transmitted infections, and reported sexual history with women. Six men reported having sexual intercourse with another man, five of whom were in the circumcision group. All six of these men also reported having sexual intercourse with women. 37 participants did not return for any subsequent visits after assessment at baseline (24 in the circumcision group and 13 in the control group) and contributed no information to the primary outcome analysis.

The median timing for the month 1 post-randomisation visit was 31 days (IQR 30–32); it was 92 days (91–93) for month 3, 184 days (182–189) for month 6, 365 days (365–371) for month 12, 549 days (547–560) for month 18, and 732 days (730–741) for month 24. There were no differences in the timing of the follow-up visits by group. The median length of follow-up was 24 months (18–24). 16 men withdrew themselves from the study before their month 24 visit: 15 (1%) in the circumcision group and one (0·1%) in the control group. The reasons given for withdrawal were: unable to come for visits (n=4), unhappy with waiting time at the clinic (5), randomised to circumcision (2), and no reason expressed (5). Withdrawals occurred between 0–1 months (n=3), 1–3 months (3), 3–6 months (3), 6–12 months (2), 12–18 months (4), and 18–24 months (1). Four men died of causes unrelated to participation in the study (two in each group), and three men (two in the circumcised group and one in the control group) were uncooperative and withdrawn by the study team. Of the 1738 participants randomised at least 24 months plus 2 weeks earlier, 1501 (86%) had completed 24 months follow-up at the time of analysis. For earlier study visits the number of follow-ups and percentages among participants reaching the time lapse since randomisation were: 2569 (92%) for month 1, 2440 (88%) for month 3, 2520 (91%) for month 6, 2474 (89%) for month 12, and 2003 (87%) for month 18. Overall, follow-up for HIV status was incomplete for 240 (8·6%) participants: 126 (9·1%) in the circumcision group and 114 (8·2%) in the control group. There were no significant differences in the event distribution with time for the missed visits. The 240 participants with incomplete information on HIV status were more likely to have some secondary education or above than the 2544 participants with complete information (76% *vs* 65%, p=0·0006). Otherwise the two groups were much the same.

Few controls (n=16, 1%) were non-adherent to treatment assignment and became circumcised during the study. Of participants randomised to circumcision, 886 (64%) had their procedures on the day of randomisation, 1116 (80%) within 1 day, 1231 (88%) within 3 days, and 1322 (95%) within 6 weeks. In total, 1334 (96%) of the participants randomised to circumcision were circumcised. There were no differences at baseline between the 69 men who did not adhere to circumcision treatment within 6 weeks of randomisation and the 1322 who did, except that 10% (7) of those who did not receive circumcision were married and living with their wife versus just 5% (64) of those who did.

During the study, seroconversion occurred in 22 participants in the circumcision group and 47 of those in the control group. The 2-year HIV incidence was 2·1% (95% CI 1·2–3·0) in the circumcision group and 4·2% (3·0–5·4) in the control group (p=0·0065); combined, it was 3·1% (2·4–3·9). Figure 2 shows the Kaplan-Meier estimates of the cumulative incidence of HIV for the 24 months of follow-up; incidence for

	Circumcision group	Control group	Overall
Demographic characteristics			
Age (years)	20 (19–22; 18–28; 1391)	20 (19–22; 17–24; 1393)	20 (19–22; 17–28; 2784)
Ethnic group			
Luo	1361 (98%)	1378 (99%)	2739 (98%)
Other	30 (2%)	15 (1%)	45 (2%)
Education level			
Less than secondary	468 (34%)	479 (34%)	947 (34%)
Any secondary or above	923 (66%)	914 (66%)	1837 (66%)
Employment status			
Employed and receiving a salary	128 (9%)	134 (10%)	262 (9%)
Self-employed	374 (27%)	355 (25%)	729 (26%)
Unemployed	889 (64%)	904 (65%)	1793 (64%)
Occupation			
Professional/managerial	25 (2%)	39 (3%)	64 (2%)
Skilled worker	141 (10%)	113 (8%)	254 (9%)
Semi-skilled worker	95 (7%)	86 (6%)	181 (7%)
Unskilled worker	698 (50%)	758 (54%)	1456 (52%)
Farm labourer/fisherman	107 (8%)	90 (6%)	197 (7%)
Student	325 (23%)	307 (22%)	632 (23%)
Marital status			
Not married (no live-in partner)	1296 (93%)	1291 (93%)	2587 (93%)
Not married (with live-in partner)	9 (0·6%)	11 (0·8%)	20 (0·7%)
Married (not living with wife)	11 (0·8%)	19 (1%)	30 (1%)
Married (living with wife)	71 (5%)	65 (5%)	136 (5%)
Physical and laboratory findings			
Weight (kg)	63 (59–68; 42–91; 1391)	62 (58–67; 40–100; 1392)	63 (59–67; 40–100; 2783)
Haemoglobin (g/L)	154 (143–163; 90–199; 1386)	153 (142–164; 83–201; 1391)	153 (142–163; 83–201; 2777)
Herpes simplex virus 2			
Positive	405 (29%)	363 (26%)	768 (28%)
Negative	980 (71%)	1029 (74%)	2009 (72%)
Syphilis			
Positive	19 (1%)	9 (0·6%)	28 (1%)
Negative	1369 (99%)	1379 (99·4%)	2748 (99%)
Trichomonas vaginalis			
Positive	27 (2%)	31 (2%)	58 (2%)
Negative	1351 (98%)	1350 (98%)	2701 (98%)
Neisseria gonorrhoeae			
Positive	32 (2%)	25 (2%)	57 (2%)
Negative	1342 (98%)	1355 (98%)	2697 (98%)
Chlamydia trachomatis			
Positive	73 (5%)	55 (4%)	128 (5%)
Negative	1300 (95%)	1325 (96%)	2625 (95%)
Haemophilus duereyi			
Positive	0 (0%)	0 (0%)	0 (0%)
Negative	21 (100%)	8 (100%)	29 (100%)
Sexual history with women			
Age at first sexual encounter (years)	16 (14–17; 5–23; 1346)	16 (14–17; 6–24; 1354)	16 (14–17; 5–24; 2700)
Sexual intercourse with any partner in previous 6 months			
Yes	1196 (86%)	1195 (86%)	2391 (86%)
No	192 (14%)	194 (14%)	386 (14%)

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Number of partners in previous 6 months			
0	192 (14%)	194 (14%)	386 (14%)
1	611 (44%)	616 (44%)	1227 (44%)
2+	585 (42%)	579 (42%)	1164 (42%)
Number of partners over lifetime	4 (3–7; 1–120; 1290)	4 (3–7; 1–390; 1303)	4 (3–7; 1–390; 2593)
Gave gifts or money to a woman for sexual intercourse in previous 6 months			
Yes	194 (16%)	210 (18%)	404 (17%)
No	1002 (84%)	985 (82%)	1987 (83%)
Drank alcohol at last time of having sexual intercourse			
Yes	142 (10%)	150 (11%)	292 (11%)
No	1248 (90%)	1239 (89%)	2487 (89%)
Used a condom at last time of having vaginal sexual intercourse			
Yes	686 (49%)	653 (47%)	1339 (48%)
No	704 (51%)	736 (53%)	1440 (52%)
Used a condom with sexual intercourse in previous 6 months			
Always	265 (22%)	254 (21%)	519 (22%)
Inconsistent	620 (52%)	632 (53%)	1252 (52%)
Never	308 (26%)	307 (26%)	615 (26%)
Last occurrence of sexual intercourse was with regular partner			
Yes	842 (80%)	826 (78%)	1668 (79%)
No	211 (20%)	227 (22%)	438 (21%)
Trouble achieving/maintaining erection in previous 6 months (participants with partner in previous 6 months)			
Yes	80 (7%)	89 (7%)	169 (7%)
No	1111 (93%)	1104 (93%)	2215 (93%)
Sexual history with men			
Ever had sexual relations with a boy or man			
Yes	5 (0·4%)	1 (0·1%)	6 (0·2%)
No	1385 (99·6%)	1388 (99·9%)	2773 (99·8%)
Injection history			
Received an injection for any reason in previous 6 months			
Yes	391 (28%)	360 (26%)	751 (27%)
No	998 (72%)	1029 (74%)	2027 (73%)

Sample sizes vary slightly from the number of randomised participants due to different data sources. Data are median (IQR; range; n) for ordinal data, or n (%) for categorical data.

Table 1: **Baseline characteristics**

intervals of follow-up are provided in table 2. The risk ratio (RR) of HIV acquisition in the circumcision group compared with the control group was 0·47 (95% CI 0·28–0·78), which corresponds to a reduction in the risk of acquiring an HIV infection in the circumcision group of 53% (22–72). The Kaplan-Meier estimates of the incidence of HIV at 12 months were 1·0% (0·5–1·6) for the circumcision group and 2·3% (1·5–3·1) for the control group (p=0·0103).

Upon further testing by PCR, three participants (two in the circumcision group and one in the control group) originally judged to be HIV positive at month 1 were found to be positive at baseline. Furthermore, one participant in the circumcision group originally deemed to be HIV positive at month 6 was confirmed as being positive at baseline. Excluding these four participants from the analysis, the 2-year HIV incidence in the circumcision group was 1·9% (95% CI 1·0–2·7) versus 4·1% (2·9–5·3) in the control group (p=0·0031); which corresponds to an RR of 0·41 (0·24–0·70), or a reduction in the risk of HIV seroconversion among circumcised men of 59% (30–76).

Excluding the participants who were confirmed HIV positive at baseline, before PCR confirmatory testing,

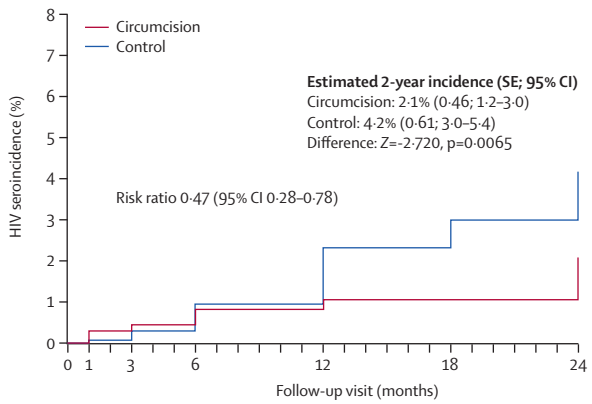
there were two HIV seroconversions in the circumcision group in the first month after randomisation and another two between months 1 and 3. Subsequent PCR testing indicated that all four were actually HIV positive at month 1; no individuals in the control group were seropositive by PCR at month 1. There were three confirmed seroconversions in the control group between month 1 and month 3, and none in the circumcision group. Thus, there were seven early seroconverters (month 1 or month 3): four in the circumcision group and three in the control group. Three of the four in the circumcision group reported no sexual activity in the month after circumcision. We cannot exclude the possibility that any of these individuals were actually HIV positive at baseline, and that their infection was not detected. Two of the three early seroconverters in the control group also denied sexual activity in the period before seroconversion. An analysis excluding the four individuals confirmed as being seropositive at baseline and the four additional early seroconverters positive at month 1 estimated 2-year HIV incidences to be 1·6% (95% CI 0·8–2·4) for the circumcision group and 4·1% (2·9–5·3) for the control group (p=0·0007). The RR was 0·32 (0·18–0·58), which corresponds to a 68% (42–82) protective effect of circumcision against HIV infection.

The as-treated analysis—which adjusted for individuals who did not adhere to the randomisation assignment—estimated the RR of circumcision to be 0·45 (95% CI 0·27–0·76). Excluding the four participants who were confirmed as being HIV positive at baseline, the RR of circumcision was 0·40 (0·23–0·68), which is equivalent to a 60% (32–77) protective effect of circumcision against HIV acquisition.

Treatment results within age strata (ages 18–20 and 21–24 years) were consistent with the overall results and there were no significant differences between the age-groups in the 2-year HIV incidence (p=0·51). For the participants who enrolled when they were 18–20 years of age, the 2-year HIV incidences were 2·5% (95% CI 1·0–3·9) in the circumcision group and 4·3% (2·6–6·1) in the control group (p=0·12). For the 21–24-year-old group, the rates were 1·7% (0·6–2·8) in the circumcision group and 4·0% (2·4–5·7) in the control groups (p=0·02). The study was not powered to detect treatment differences within the two age-groups.

After adjustment for baseline variables for which there seemed to be differences between the two study groups at baseline, only infection with herpes simplex virus 2 at baseline was found to be associated with HIV incidence (RR 1·91, 95% CI 1·18–3·08). The treatment effect remained strong with all adjustments that were considered, and the adjusted RR varied between 0·44 and 0·47.

Not all circumcised men adhered to the 30-day period of post-circumcision abstinence. 60 participants (4·5%) in the circumcision group reported having had sexual intercourse before 30 days post-circumcision, including one of the early seroconverters (month 1) noted above, and another whose HIV infection was detected at the month 6 visit. Both of these participants had adhered to treatment.



Circumcision (n=1391)						
Number at risk	1367	1351	1323	1287	1029	764
Number HIV positive	4	2	5	3	0	8 (22 total)
Control (n=1393)						
Number at risk	1380	1368	1350	1302	1035	740
Number HIV positive	1	3	9	18	7	9 (47 total)

Figure 2: **Cumulative HIV seroincidence across follow-up visits by treatment**
Time to HIV-positive status is taken as the first visit when a positive HIV test result is noted. Time is credited as the follow-up visit month. Participants without HIV-positive status are censored at the last regular follow-up visit completed where HIV testing was done, credited specifically as months 1, 3, 6, 12, 18, and 24.

	Circumcision group	Control group	Total
0–6 months*	0·8% (0·3–1·3)	1·0% (0·4–1·5)	0·9% (0·5–1·2)
6–12 months†	0·2% (0·1–0·7)	1·4% (0·8–2·2)	0·8% (0·5–1·3)
12–18 months†	0·0% (0·0–0·5)	0·7% (0·3–1·5)	0·3% (0·1–0·7)
18–24 months†	1·0% (0·5–2·1)	1·2% (0·6–2·4)	1·1% (0·7–1·8)
0–24 months*	2·1% (1·2–3·0)	4·2% (3·0–5·4)	3·1% (2·4–3·9)

Data are % (95% CI). *Based on Kaplan-Meier methods. †Based on the number of new incidents of HIV infection detected for the interval divided by the number of participants at risk during the interval.

Table 2: **Incidence rates for intervals of follow-up**

All but one of the 1334 men who were circumcised returned for their 3-day postsurgical visit, and all but six returned after 8 days. All those employed had resumed working by the 3-day visit. Among all men circumcised, 1287 (96%) reported having returned to normal activities by the 3-day visit, and all but one person had returned to normal activities by the 8-day visit. At the 3-day visit, 643 (48%) reported no pain, 690 (52%) reported very mild pain, and none reported mild to severe pain. By the 8-day visit, 1179 (89%) reported no pain, and 148 (11%) reported very mild pain. Of the 1334 men circumcised, 1281 (96%) had a 30-day postsurgical wound examination. The wound was judged to be completely healed in all but 16 (1%) individuals. All had returned to normal general activities. All wounds were completely healed by the month 3 visit. 1274 (99·5%) individuals were "very satisfied" and six (0·5%) were "somewhat satisfied" with their circumcision; one

	Number of occurrences	Severity	Related to surgery?
Bleeding	5	2 mild, 3 moderate	Definitely
Infection	5	2 mild, 3 moderate	Definitely
Disruption	4	Mild	Definitely
Delayed healing	3	Mild	Definitely
Swelling	2	1 mild, 1 moderate	Definitely
Anaesthetic-related event	1	Moderate	Definitely
Wound at base of penis	1	Moderate	Probably
Pubic abscess	1	Moderate	Possibly
Folliculitis	1	Mild	Possibly
Erectile dysfunction	1	Moderate	Possibly

Table 3: **Adverse events recorded by severity and relatedness to the surgery**

person was "somewhat dissatisfied", and none were "very dissatisfied". The somewhat dissatisfied participant reported weak erections at his month 1 visit, but this complaint resolved at subsequent visits and he was sexually active.

Table 3 summarises the 24 adverse events recorded as possibly, probably, or definitely related to circumcision that occurred in 23 (1·7%, 95% CI 1·1–2·6) of the 1334 participants. Postoperative bleeding (n=5) and infections (5) were the most common adverse events; wound disruptions (4), delayed healing (3), and swelling at the incision site (2) were also recorded more than once. There was an anaesthetic-related event when a participant had a generalised convulsion, possibly triggered by excessive use of local anaesthetic combined with hypoglycaemia, since the patient had not eaten for 36 hours before the surgery. Thereafter, our surgical protocol was modified to restrict the amount of local anaesthetic used. 21 adverse events among 20 participants (1·5%, 95% CI 0·9–2·3) were probably or definitely related to surgery. All were mild or moderate in severity. None was judged to be severe, and, except for the case of erectile dysfunction, all adverse events resolved with treatment within hours or days. We note that erectile dysfunction was reported post-randomisation in both study groups, with an incidence of 1·5% in the circumcision group and 1·0% in the control group (p=0·24).

10 154 unrelated adverse events were recorded among 1979 (71%) participants. The most frequent unrelated adverse events were upper respiratory tract infections (3189 events, 1184 participants, 43%), malaria (2271 events, 1076 participants, 39%), skin or mucous membrane infections (1011 events, 682 participants, 24%), and gastroenteritis (456 events, 327 participants, 12%). Study groups did not differ with respect to these common illnesses. There were 32 severe adverse events and four deaths, all unrelated to participation in the study. Severe adverse events were those that resulted in hospitalisation and consisted mostly of trauma due to traffic or work-related accidents, and to severe malaria and tuberculosis. There were 17 severe adverse events in 16 participants in the circumcision group and 15 severe adverse events in 14 participants in the control group. Deaths were due to traffic injuries (n=2), shooting by police (1), and beating by thugs (1), with two deaths in the circumcision group and two in the control group. Men in the control group had higher frequencies of abdominal or gastrointestinal conditions (p=0·047) and, as expected, of balanitis, phimosis, or paraphimosis (p<0·0001) than did those in the circumcision group.

	Circumcision group	Control group	p value*
Unprotected sexual intercourse with any partner in previous 6 months (p=0·1666†)			
Baseline	867/1385 (63%)	872/1387 (63%)	
Month 6	623/1231 (51%)	623/1262 (49%)	
Month 12	631/1227 (51%)	585/1228 (48%)	
Month 18	505/985 (51%)	495/988 (50%)	
Month 24	381/741 (51%)	331/727 (46%)	0·0349
Last time had sexual relations with a casual partner (p=0·8044†)			
Baseline	211/1053 (20%)	227/1053 (22%)	
Month 6	180/929 (19%)	192/955 (20%)	
Month 12	199/1014 (20%)	204/1007 (20%)	
Month 18	198/985 (20%)	196/988 (20%)	
Month 24	140/741 (19%)	125/729 (17%)	0·2174
Sexual abstinence in previous 6 months (p=0·4287†)			
Baseline	192/1388 (14%)	194/1389 (14%)	
Month 6	191/1232 (16%)	216/1263 (17%)	
Month 12	188/1227 (15%)	203/1229 (17%)	
Month 18	155/985 (16%)	166/988 (17%)	
Month 24	104/741 (14%)	132/728 (18%)	0·0825
Consistent condom use in previous 6 months (p=0·1143†)			
Baseline	265/1193 (22%)	254/1193 (21%)	
Month 6	370/1040 (36%)	378/1046 (36%)	
Month 12	358/1039 (34%)	398/1025 (39%)	
Month 18	296/830 (36%)	304/822 (37%)	
Month 24	231/637 (36%)	246/595 (41%)	0·0326
Two or more partners in previous 6 months (p=0·0383†)			
Baseline	585/1388 (42%)	579/1389 (42%)	
Month 6	409/1232 (33%)	443/1263 (35%)	
Month 12	360/1227 (29%)	408/1229 (33%)	
Month 18	294/985 (30%)	300/988 (30%)	
Month 24	225/741 (30%)	199/728 (27%)	0·2044

Data are n/N (%). *Test for difference between the treatment groups in change from baseline to month 24. †Global test for any differences between the treatment groups in changes from baseline to follow-up visits.

Table 4: **Sexual history with women reported at baseline and follow-up visits**

Five behavioural variables were selected a priori for detailed analysis of changes in HIV risk behaviour by treatment group (table 4). From baseline to month 6, circumcised and uncircumcised participants both reported safer sexual behaviours in absolute terms, with a lower proportion of men reporting unprotected sexual intercourse with any partner, sexual intercourse

with a casual partner at the last time of such relations, and having two or more sexual partners in the previous 6 months. Similarly, the proportion of men practising sexual abstinence and using a condom consistently during the previous 6 months rose from baseline to month 6. These gains were sustained for the duration of the 24 months of follow-up, with the exception of sexual abstinence in the circumcision group, which returned to baseline level at month 24.

There was little difference between circumcised and uncircumcised men in change in sexual behaviour measures across the follow-up visits, with the exception of two or more partners in the previous 6 months (p=0·0383). There was a linear decrease across visits in the proportion of men in the control group reporting two or more partners in the previous 6 months, whereas the proportion reporting the same behaviour in the circumcision group fell from month 0 to month 6 and remained fairly stable thereafter. Focusing on change specifically from baseline to month 24, differences between the study groups were found for unprotected sexual intercourse (p=0·0349) and consistent condom use (p=0·0326), with individuals in the control group practising the safer sexual behaviours (table 4). Notably greater proportions of circumcised men reported riskier behaviours on all of the other three behavioural variables at month 24, although the differences were small and not significant.

Discussion

Our results confirm that male circumcision substantially reduces the risk of acquiring an HIV infection. Circumcision provided a 53% (95% CI 22–72) protective effect against HIV acquisition compared with the control group and a 60% (32–77) protective effect after adjustments for non-adherence and for those individuals who were found to be HIV positive at baseline. These findings are much the same as those from the Orange Farm trial in South Africa (60% [32–76] protection against HIV infection, with a larger reduction of 76% [56–86] found in a per-protocol analysis that adjusted for crossovers)[21] and to the recently announced 51% protective effect found in Rakai, Uganda.[35] All three trials testing the efficacy of male circumcision against HIV acquisition in African men were stopped by their data and safety monitoring boards before their designed completion because of significant reductions in HIV incidence in the circumcision groups, making it unethical to continue following control group participants without offering them circumcision. Finding a causal relation between HIV infection and male circumcision is consistent with the reductions in HIV prevalence found in meta-analyses of observational studies[14,24] and with investigations of the immunohistochemistry of foreskin tissue.[16–18] Such consistency of clinical, observational, and biological data has not been reported for any other intervention that addresses reduction of HIV incidence in adults.

There was a difference of 7% (53% *vs* 60%) in the estimated protective effect of circumcision against HIV infection between the intention-to-treat analysis and the as-treated analysis, which accounted for men who did not adhere to treatment and those confirmed seropositive at baseline. Although the conclusions from the two analyses are the same, the two measures of effect size should be considered in the context of an increased effect of male circumcision on HIV prevalence at the population level. For planning purposes, the 60% protective effect probably represents the more accurate estimate of the treatment effect, since it compares truly circumcised HIV-negative men to truly uncircumcised HIV-negative men post-randomisation. Recent simulation models based on the assumption of a 60% protective effect of circumcision estimate that as many as 2 million new HIV infections and 300 000 deaths could be averted over the next 10 years in sub-Saharan Africa, assuming 100% uptake of male circumcision. Over the next 20 years, these numbers could amount to 3·7 million and 2·7 million, respectively.[36] Other models, also based on a 60% protective effect, estimate that HIV prevalence could be reduced by half to two-thirds (depending upon the level of uptake of male circumcision) in currently high prevalence areas, including Nyanza Province, Kenya, where this study was done (unpublished data). Furthermore, based on 2005 conditions in Gauteng Province, South Africa, male circumcision would be highly cost-effective, saving about $2·4 million over 20 years per 1000 circumcisions.[37]

This study showed that medical circumcision can be provided safely to adult men in a developing country setting. Adverse event rates were comparable with rates documented for neonatal circumcision in developed countries.[38–40] Currently, rates of complications in clinical settings in Africa are poorly documented, but could vary between 2% to as high as 17·5%.[41–43] The 1·5% rate of adverse events in our study was lower than the 3·6% rate in Orange Farm.[21] Both studies used much the same forceps-guided method.[28] The difference in rates could be a result of multiple factors: all procedures in Kisumu were done at our study clinic by our own, highly trained and experienced practitioners; we had regular surgical case conferences to review outcomes; participants were given clear written postoperative instructions; and participants had scheduled clinic visits 3, 8, and 30 days after the procedure. The Orange Farm trial contracted experienced local private practitioners to do the operations in their own offices, and patients were seen only if they came back with a complication. The Orange Farm trial might more closely resemble what the situation is likely to be under non-study conditions. Our results indicate that extensive training, proper instrumentation, clear postoperative instructions, and continuing quality assurance and control are helpful to assure optimum outcomes.[28,44] These lessons will be important for implementation of wide-scale medical male circumcision interventions.

If circumcised men believe that they are protected from HIV infection, there is a possibility that they will compensate for their perceived risk reduction by engaging in higher risk behaviours. A moderate level of risk compensation could mitigate any benefit of circumcision in preventing HIV infections. Some observational studies have found that circumcised men engage in higher risk behaviours than uncircumcised men,[45,46] and the Orange Farm trial found that circumcised men had slightly higher levels of risk, as measured by five behavioural factors.[21] However, a prospective cohort study in Siaya and Bondo districts, near the site of our trial, found no increase in risky sexual acts by men after circumcision compared with uncircumcised controls.[47] Our study documented a reduction in risk behaviours in both circumcised and uncircumcised participants from baseline to follow-up, indicating that the initial behavioural counselling and voluntary HIV testing offered to the participants were effective. During follow-up visits as a whole, there were no significant differences between circumcised and uncircumcised men in change of the measured sexual behaviours, except in the proportion of men having two or more sexual partners, which showed a progressive decline in the control group; in the circumcision group, the proportion remained stable after month 6. Circumcised men exhibited slightly riskier behaviour on all five assessed measures at month 24 and this was significant for two of the measures—unprotected sexual intercourse with any partner in the previous 6 months and consistent condom use—at that time point. However, the differences between the two groups are attributable to increases in safer sexual practices in the control group rather than to riskier behaviour patterns in the circumcision group, indicating that risk compensation[48] (ie, behavioural disinhibition) did not occur during the 24 months of this study. The reasons men in the control group might have decreased their HIV risk behaviours more than those in the circumcision group are speculative, but could be due to changes in the Kisumu community, differential counselling by study staff, or a perception that being uncircumcised puts one at greater risk. Whether the differences in risk behaviours persist after 24 months remains to be seen. We will continue to follow the cohort to observe behavioural changes as well as HIV seroconversion rates for as long as 5 years after randomisation.

All men in the circumcision group were counselled to refrain from masturbation and sexual activity for at least 30 days after surgery. However, 60 of 1334 (4%) failed to abstain by their own report. Of these 60 men, two seroconverted during their study participation—one at month 6 and the other at month 1. The month 1 seroconverter could have become infected with HIV through sexual activity before his surgical wound had fully healed. There were three other circumcised participants who denied being sexually active in the first month after surgery, but who seroconverted after 1 month. These findings reinforce the importance of developing effective counselling techniques to promote abstinence from sexual activity for at least the first month after circumcision.

There were several limitations to this study. Medical workers could not be blinded to treatment. However, non-medical staff who did HIV tests, administered questionnaires, and counselled participants about risk reduction were blinded to treatment, although some participants divulged their circumcision status during counselling. Questions directly relevant to circumcision status were asked by medical staff only. Measurement of behavioural risk compensation relied on self-report, which could result in under or over-reporting; however, there is no a priori expectation for the direction in which this might occur, nor any suggestion that this should differ between treatment groups. Some participants did not report for all scheduled study visits. HIV test results were incomplete for 9% of the participants; however, there were no baseline differences between those with complete follow-up for HIV status and those without. With such a low frequency of missed visits and an annual HIV seroincidence of 1·6%, any undetected HIV infections would have had little effect on the study results. Moreover, unlike interventions with repeated treatment, often unseen by the study staff, adherence to the intervention was known, and when men missed a visit they were probably protected by circumcision to the same degree as those who did not miss a visit.

Circumcision technique represents one possible source of variation in the protective effect of male circumcision. Although the Orange Farm trial and this study used similar forceps-guided methods,[28] the amount of foreskin tissue remaining after the procedure could vary, depending on the operator. The protective effect of circumcision against HIV infection is thought to derive in part from postsurgical development of a layer of keratinised squamous epithelial cells that limit viral entry to underlying HIV target cells.[16,18] How long it takes the residual tissue to fully heal and become keratinised has not been studied. Our surgical protocol called for retention of 1–1·5 cm of residual inner foreskin. Although the results from the three trials are remarkably consistent, differences in effect sizes could be a result of differences in surgical technique and healing time.

Generalisability of our study results to other populations could be restricted by several factors. The surgical conditions were near optimum, and postoperative wound checks were frequent. Participants were screened to exclude those who were HIV seropositive, who had symptomatic illnesses, or contraindications to surgery. In standard public-health settings, HIV testing might not always be practical or acceptable. Further, if circumcision proves partly protective against HIV transmission to sexual partners, as is now being tested in Uganda, then circumcising HIV-infected men could become a priority. We enrolled only men who were aged 18–24 years, and

almost all were sexually active within the previous year. Ideally, if introduced widely, this intervention will be made available to younger males before they become sexually active. The participants in this study had frequent contact with study staff. They had free medical care, were counselled about safe sexual practices, had unrestricted access to condoms, were tested for sexually transmitted infections, and were treated for bacterial infections. This level of contact, intense counselling, and medical care is unlikely to pertain in standard settings. Finally, almost all the participants in this study identified as belonging to the same ethnic group—the Luo. If Luo males engage in systematically different behaviours from men of other ethnic groups, the results of this study might not apply to other regions of Africa. However, this seems unlikely, since our results are very similar to those from other clinical trials and observational studies, and there is no reason to suspect that Luo men act differently from others in response to circumcision.

Although there is little evidence of risk compensation by the circumcised men in this study, beliefs and attitudes about circumcision could change substantially after the results of the three clinical trials are widely publicised and interventions are put in place to promote male circumcision. A challenge to prevention specialists and clinicians will be to develop circumcision interventions that communicate the benefits of the procedure, while also explaining that circumcision does not offer full protection from HIV acquisition. 13 studies in nine sub-Saharan African countries found that between 29% and 80% of men in traditionally non-circumcising communities would prefer to be circumcised if the procedure could be offered safely, with the minimum of pain, and at low cost.[49] Now that compelling evidence is available that male circumcision reduces risk of HIV acquisition, expectations about the effectiveness of the procedure and demand could increase dramatically, perhaps burdening health facilities and opening opportunities for under-qualified, poorly equipped practitioners with little training in HIV prevention counselling.[50] Circumcision will be most effective if it is not perceived as a stand-alone clinical procedure, but as one component of a full suite of HIV prevention and reproductive health services, including HIV testing and counselling, diagnosis and treatment of sexually transmitted infections, condom promotion, behavioural change counselling and promotion, and other methods as they are proven effective. With commitment to proven prevention methods today, there is the possibility of turning around the HIV epidemic.

Contributors

R C Bailey participated in conceptualising the study, designing the protocol and study instruments, providing scientific and management leadership, reviewing study data, drafting the manuscript and coordinating submission. S Moses participated in conceptualising the study, designing the protocol and study instruments, providing medical, scientific and management leadership, reviewing and analyzing study data, and drafting and editing the manuscript. C B Parker participated in revising the protocol and study instruments, managed and coordinated data input, review and quality control, did the bulk of the data analyses, drafted substantial sections of the manuscript, and reviewed and edited the entire manuscript. K Agot participated in designing the protocol and study instruments, managed and coordinated every aspect of the study operations, ensured outreach to the study community, reviewed and corrected study data, and reviewed and edited the manuscript. I Maclean participated in the design of the protocol and study instruments, established the laboratory and all lab protocols, oversaw management of the laboratory, reviewed study data, and reviewed and edited the manuscript. J N Krieger participated in the design of the protocol and study instruments, trained clinicians and oversaw surgical procedures, reviewed study data, and reviewed and edited the manuscript. C F M Williams participated in review of the protocol and revision of study instruments, provided scientific leadership, and reviewed and edited the manuscript. R T Campbell participated in the analysis of the behavioural study data, and reviewed and edited the manuscript. J O Ndinya-Achola participated in designing the protocol and study instruments, provided overall medical, scientific and management leadership, assisted with study operations, liaised with local, national and university partners, and reviewed and edited the manuscript.

Conflict of interest statement

We declare that we have no conflict of interest.

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VIEWPOINTS ON HIV RESEARCH

Male circumcision for the prevention of heterosexually acquired HIV infection: a meta-analysis of randomized trials involving 11 050 men*

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Objectives

Observational studies and a small collection of randomized controlled trials (RCTs) suggest that male circumcision may significantly reduce HIV transmission between sero-discordant contacts. The Joint United Nations Programme on HIV/AIDS (UNAIDS) and World Health Organization have recently announced recommendations to scale up male circumcision in countries with generalized epidemics and low levels of male circumcision. However, no meta-analysis has been conducted to determine the effectiveness of this intervention.

Methods

We conducted a systematic review of medical literature, and included any RCTs assessing male circumcision to prevent heterosexually acquired HIV infection among males. We used the DerSimonian–Laird random effects method to pool study outcomes. We calculated the relative risk (RR), risk difference, number needed to treat (NNT) and I^2, all with 95% confidence intervals (CIs).

Results

We identified three RCTs that met our inclusion criteria, involving a total of 11 050 men. The pooled RR was 0.44 (95% CI 0.33–0.60, $P<0.0001$, $I^2 = 0\%$, 95% CI 0–35%). The risk difference was 0.014 (95% CI 0.07–0.21), yielding a NNT of 72 (95% CI 50–143).

Conclusions

Male circumcision is an effective strategy for reducing new male HIV infections. Its impact on a population level will require consistently safe sexual practices to maintain the protective benefit.

Keywords: circumcision, HIV/AIDS, meta-analysis, prevention

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Introduction

In 2007, there were an estimated 33.2 million (plausibility range 30.6–36.1) people living with HIV/AIDS globally, with some 22.5 million (range 20.9–24.3) of these adults and children living in sub-Saharan Africa. We have every reason to believe that this figure will continue to increase unless effective interventions can slow the progress of the epidemic [3]. In March 2007, the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization announced recommendations to scale up male circumcision in countries affected by generalized epidemics that currently have low levels of male circumcision [4]. Male circumcision represents an important intervention in combating HIV/AIDS: systematic reviews of observational studies have indicated the important protective effects of this intervention [5,6]. Recently, the first randomized clinical trials were completed. We have conducted the first meta-analysis of the recently completed randomized trials.

*See editorial by Lazarus *et al.* [1] on pp. 327–328 and article by Rice *et al.* [2] on pp. 329–331 in this same issue.

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Methods

We included any randomized trial assessing male circumcision to prevent heterosexually acquired HIV infection

among males. We systematically reviewed the medical literature and searched electronic databases (MedLine, EMBASE, CINAHL), electronic conference websites (IAS, CROI) and clinical trial registries (Clinicaltrials.gov, Meta-Register). The completed trials have been well publicized and we contacted the study authors. We abstracted data independently, in duplicate, addressing trial setting, participants, trial duration and number of infections in each group (active and controls). Additionally, we abstracted data on methods of allocation concealment, randomization and adherence to the intention-to-treat principle.

For meta-analysis, we used the DerSimonian–Laird random effects method to pool the study outcomes. This method recognizes and anchors studies as a sample of all potential studies, and incorporates an additional between-study component to the estimate of variability [7]. Our primary endpoint was the number of patients seroconverting. We excluded patients who were HIV-positive at baseline but were still randomized [8]. We calculated the relative risk, risk difference and number needed to treat (NNT), all with 95% confidence intervals (CIs). Baseline risks across the groups were similar (1.78–4.2% over 24 months). We also calculated the I^2 statistic as a measure of the proportion of the overall variation that was attributable to between-study heterogeneity, with its 95% CI [9]. We used Comprehensive Meta-Analysis Version 2.1 (Biostat Inc., Englewood, NJ, USA) and StatsDirect Version 2 (StatsDirect Ltd, Manchester, UK) for all calculations.

Results

We identified three randomized trials that met our inclusion criteria [8,10,11]. Table 1 presents the study characteristics and individual study outcomes. All studies adequately reported the *a priori* determined methodological issues. All trials stopped prior to complete enrolment because of Data Safety and Monitoring Board recommendations of clear effectiveness.

Our pooled analysis indicates a RR of 0.44 (95% CI 0.33–0.60, $P \leq 0.0001$, $I^2 = 0\%$, 95% CI 0–35%; see Fig. 1) in favour of circumcision, corresponding to a RR reduction of 56% (95% CI 40–67%). The risk difference is 0.014 (95% CI 0.07–0.21), yielding a NNT of 72 (95% CI 50–143).

Discussion

Our meta-analysis should be of interest to policy-makers, clinicians and the public. There is a large and consistent effect of circumcision in the prevention of heterosexually acquired HIV infection. As new prevention technologies prove challenging, circumcision appears to be an inexpensive and effective prevention strategy. Challenges will now exist in expanding access to circumcision and addressing cultural concerns about the acceptability of the intervention [12].

There are several strengths and limitations to consider in our analysis and in the included trials. Strengths of our review include our extensive searching, contact with

Table 1 Study characteristics and outcomes

Study	Design	Setting	Population	*n*	Outcomes		Relative risk (95% confidence interval)
					Intervention	Control	
Auvert *et al.* (2005)*	Randomized trial	Orange farm, South Africa	Males aged 18–24 years	3128	20/1546	49/1582	0.42 (0.25–0.70)
Bailey *et al.* (2007)**	Randomized trial	Kisumu, Kenya	Males aged 18–24 years	2780	19/1388	46/1392	0.41 (0.24–0.70)
Gray *et al.* (2007)	Randomized trial	Rakai district, Uganda	Males aged 15–49 years	4996	22/2474	45/2522	0.50 (0.30–0.83)

*3274 randomized, 3128 included in analysis.
**2784 randomized, 2780 included in analysis.

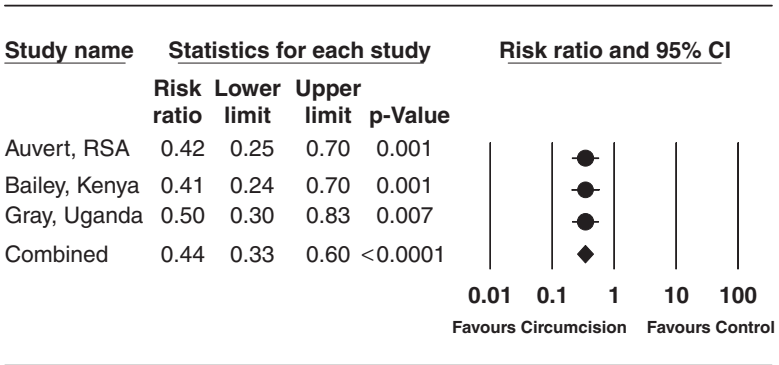

Fig. 1 Random effects meta-analysis.

authors and policy-makers, and use of the random-effects model to provide a more conservative estimate. Limitations of our review include our inability to conduct sensitivity analysis because of the low number of trials included. However, given the consistency of findings, it is unlikely that any trial-level characteristics influenced the outcomes. Our meta-analysis used binary estimates. It is possible that our results would change marginally had we used time-to-event data.

Limitations of the trials also exist. All trials were stopped early; it is likely that, because of stopping early, results represent an over-estimate of the true reduction in infection rates [13,14]. However, the magnitude of the estimates is so great, and the results so consistent, that this limitation does not threaten the inference that, when administered to similar populations in a similar fashion, circumcision results in an appreciable RR reduction.

Participants in these trials received education to reduce their likelihood of infection, including safe-sex counselling and the recommendation of abstinence during the healing period. It is possible that this education will impact the generalizability of the trials because it may reduce the number of exposures that participants have in comparison to the general population. The trials were unable to blind participants to the intervention and control and, as a result, some participants in the control groups received the intervention outside of the trial. To counter this effect, the investigators also performed a per-protocol analysis and found a similar magnitude of effect.

Media attention has focused on the '60%' reduction in infections observed initially in the trial by Auvert *et al.* [11]. However, our pooled analysis indicates that the protective effect of male circumcision may be somewhat different and that the actual population effect of the intervention may be less compelling. The NNT of 72 (95% CI 50–143) suggests that approximately 72 circumcisions will have to be conducted over a 2-year period to prevent a new infection, although this will differ in populations with varying baseline risks. Researchers have modelled the cost-effectiveness and population impact of widespread circumcision. Recent models assessing circumcision in South Africa and Uganda found savings of US$2411 and US$2631 per infection averted, respectively [15,16]. While the costs of the surgery will vary by country, the lives saved from prevented infections and prevented antiretroviral provision are likely to overwhelm the costs of the brief surgery.

A number of considerations suggest that the effect seen in the trials may not be reproduced with widespread dissemination of the intervention. With circumcision, this is observed as often longer than 6 weeks [17]. Men who engage in sex during the healing period may place themselves at increased exposure to infection. Counselling patients on the abstinence period may prove to be a challenge for successful implementation.

In addition, circumcised men may have an exaggerated sense of protection from sexually transmitted diseases including HIV that could influence their behaviour. Currently, we do not know how circumcision will impact upon behaviours; however, a modelling study from Uganda indicated that an increased number of sexual partners will counteract the beneficial impact of circumcision [16].

This situation raises important considerations for the conduct of preparedness studies addressing new prevention strategies. Despite the plethora of preparedness studies assessing potential HIV vaccines, comparatively few studies have addressed circumcision. While vaccines remain elusive, circumcision now represents an important tool in preventing new infections. As more circumcision data are developed there will be greater pressure on ministries to promote circumcision, potentially challenging local traditions. For interventions to have an important population-level effect, there is a need for local buy-in. Assessing cultural acceptability and strategies to overcome barriers will represent one of the greatest challenges for prevention strategies this year.

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