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# Foreskin surface area and HIV acquisition in Rakai, Uganda (size matters)

**Godfrey Kigozi**[a], **Maria Wawer**[b], **Absalom Ssettuba**[a], **Joseph Kagaayi**[a], **Fred Nalugoda**[a], **Stephen Watya**[d], **Fred Wabwire Mangen**[c], **Noah Kiwanuka**[c], **Melanie C. Bacon**[e], **Tom Lutalo**[f], **David Serwadda**[c], and **Ronald H. Gray**[b]

[a]Rakai Health Sciences Program, Entebbe, Uganda [b]Johns Hopkins University, Bloomberg School of Public Health, Baltimore, Maryland, USA [c]Makerere University School of Public Health, Kampala [d]Urology Unit, Department of Surgery, Mulago Hospital, Makerere University, Kampala, Uganda [e]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA [f]Uganda Virus Research Institute, Entebbe, Uganda.

## Abstract

**Introduction—**Male circumcision reduces HIV acquisition in men. We assessed whether foreskin surface area was associated with HIV acquisition prior to circumcision.

**Methods—**In two randomized trials of male circumcision, the surface area of the foreskin was measured after surgery using standardized procedures. Nine hundred and sixty-five initially HIV-negative men were enrolled in a community cohort who subsequently enrolled in the male circumcision trials, provided 3920.8 person-years of observation prior to circumcision. We estimated HIV incidence per 100 person-years prior to circumcision, associated with foreskin surface area categorized into quartiles.

**Results—**Mean foreskin surface area was significantly higher among men who acquired HIV (43.3 $cm^2$, standard error 2.1) compared with men who remained uninfected (36.8 $cm^2$, standard error 0.5, $P = 0.01$). HIV incidence was 0.80/100 person-years (8/994.9 person-years) for men with foreskin surface areas in the lowest quartile ( ≤26.3 $cm^2$), 0.92/100 person-years (9/975.3 person-years) with foreskin areas in the second quartile (26.4–35.0 $cm^2$), 0.90/100 person-years (8/888.5 person-years) with foreskin area in the third quartile (35.2–45.5 $cm^2$) and 2.48/100 person-years (23/926.8 person-years) in men with foreskin surfaces areas in the highest quartile (>45.6 $cm^2$). Compared with men with foreskin surface areas in the lowest quartile, the adjusted incidence rate ratio of HIV acquisition was 2.37 (95% confidence interval 1.05–5.31) in men with the largest quartile of foreskin surface area.

**Conclusion—**The risk of male HIV acquisition is increased among men with larger foreskin surface areas.

### Keywords

foreskin surface area; HIV acquisition; HIV target cells; male circumcision

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Correspondence to Dr Godfrey Kigozi, MD, MPH, Rakai Health Sciences Program, P.O. Box 279, Kalisizo, Uganda. Tel: +256 772593267; fax: +256 48122153; gkigozi@rhsp.org, gkigozi@imul.com.
R.H.G., D.S., F.W.M., M.J.W., G.K. and S.W. designed and conducted the randomized trials, which provided data for this analysis. G.K., A.S. and R.H.G. designed the analysis. G.K., F.N., J.K., T.L. and N.K. collected data or did coordination for the study. G.K., A.S. and R.H.G. analyzed the data. All authors contributed to writing of the manuscript.

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## Introduction

Several observational and ecological studies [1–5] and three randomized clinical trials [6–8] have shown that male circumcision reduces the risk of male HIV acquisition by 50–60%. Male circumcision is now recommended by WHO/United Nations Joint Programme on HIV/AIDS (UNAIDS) as an HIV prevention strategy [9].

A number of hypotheses have been advanced to explain the biological plausibility of these findings. Male circumcision reduces the rates of genital ulcer disease (GUD) [8,10,11], and GUD may provide a portal of entry for HIV. The moist subpreputial environment may favor survival of HIV, thus increasing HIV entry following exposure to infected genital fluids. Also, the foreskin is retracted over the shaft during intercourse exposing the lightly keratinized inner mucosa to vaginal/rectal secretions and to microtears, particularly at the frenulum [11]. The foreskin also contains Langerhans/dendritic cells and CD4/CD8 T lymphocytes, which are target cells for HIV infection [12–15]. Male circumcision removes these vulnerable tissues, so the only remaining unkeratinized mucosa is the urethral meatus, which presents a smaller surface area for infection.

As there is evidence that the foreskin increases vulnerability to HIV, we hypothesized that the size of the foreskin might be related to the risk of HIV infection. Therefore, we assessed the association between the surface area of the foreskin measured at time of male circumcision and the risk of HIVacquisition in uncircumcised men prior to their surgery.

## Methods

The study was conducted in Rakai, Uganda. Men eligible for this retrospective cohort study were uncircumcised men aged 15–49 years who were initially HIV-negative participants in the Rakai Community Cohort Study (RCCS) described below. These men were subsequently enrolled into the randomized trials of male circumcision and had measurement of their foreskin surface area following surgery. There were 965 initially HIV-negative men under surveillance in the RCCS for a maximum of 4 years prior to surgery. We determined HIV acquisition in these men and assessed the association between foreskin size measured after surgery and the incidence of HIV acquisition while under surveillance prior to male circumcision.

The RCCS is an open cohort with an annual follow-up of approximately 12 000 adults residing in Rakai district who provided written informed consent at enrollment and at each follow-up visit. At each visit, participants provided information on social–demographic characteristics and sexual behaviors. Samples including venous blood for HIV testing were collected. Voluntary HIV counseling and testing, health education on HIV prevention strategies [abstinence, be faithful and use condoms (ABC)] and free or subsidized condoms were provided at every visit.

We conducted two randomized trials of male circumcision for HIV prevention in men enrolled between 2003 and 2006. The trials have been described elsewhere [8,16]. Men in both trials were randomized to either receive male circumcision immediately or have male circumcision delayed for 2 years. Informed consent and a questionnaire were administered at baseline and follow-up at 6, 12 and 24 months. Thus, trial participants who had previously been enrolled in the Rakai cohort study afforded an opportunity for assessment of HIV incidence during cohort surveillance in relation to the foreskin surface area measured after male circumcision.

Both the RCCS and the male circumcision trials were approved by four institutional review boards: the Science and Ethics Committee of the Uganda Virus Research Institute (Uganda), the National Council for Science and Technology (Uganda), the Committee for Human Research at Johns Hopkins University Bloomberg School of Public Health (USA) and the Western Institutional Review Board (USA). The trials were registered with Clinical.Trials.Gov numbers NCT00425984 and NCT00124878.

HIV status was determined using two enzyme immunoassays (EIA): Veronostica HIV-1 (Organon Teknika, Charlotte, North Carolina, USA) and Welcozyme HIV-1 and HIV-2 (Murex Diagnostic, Dartford, UK). Concordant positive EIA tests were interpreted as HIV positive, and concordant negative EIA tests were interpreted as HIV negative. Discordant EIA tests and all incident HIV cases were confirmed by western blot (Calypte Biomedical Corp., Rockville, Mississippi, USA). Men who tested HIV positive were referred to the Rakai President's Emergency Plan for AIDS Relief (PEPFAR)-funded HIV care and treatment program.

Male circumcision was conducted by trained physicians using the sleeve circumcision method, which has been described elsewhere [8]. Measurement of foreskin surface area was done by physicians immediately after completion of male circumcision. The foreskin was placed on a flat surface, and mild tension was applied at all four corners of the foreskin using artery forceps to straighten the edges. A tape calibrated in centimeters was used to measure the length and width of the foreskin at the midpoint, and results were recorded.

### Statistical analysis

Person-time for estimation of HIV incidence was accrued from the date of enrollment into the Rakai cohort until the date of seroconversion or male circumcision. The timing of HIV infection was estimated at the midpoint between the first HIV-positive and last HIV-negative serologic test. There were no losses to follow-up, as we purposefully selected initially HIV-negative men who were under continuous observation until time of surgery.

Foreskin surface area in square centimeters ($cm^2$) was calculated by multiplying the length by the width of the foreskin. Mean and median foreskin surface areas were stratified by age and by HIVacquisition or persistent HIV-negative status. The *t*-test was used to assess differences in the mean foreskin surface areas by age and between men who seroconverted and those who remained uninfected. Foreskin surface area was also categorized into quartiles [ ≤25% (7.0–26.3 $cm^2$), 26–50% (26.4–35.0 $cm^2$), 51–75% (35.1–45.5 $cm^2$) and >75% (45.6–99.8 $cm^2$)]. We then assessed HIV incidence by quartiles of foreskin surface area. Incidence rate ratios (IRRs) and 95% confidence intervals (95% CIs) of HIV acquisition associated with quartiles of foreskin surface area were estimated by Poisson multiple regression after adjustment for age, education, religion, number of sex partners and condom use.

The analyses for this study used Stata version 8.0 (StataCorp Inc., College Station, Texas, USA).

## Results

Nine hundred and sixty-five men were included in this analysis, of whom 48 seroconverted prior to male circumcision (Table 1). The median foreskin surface area was larger among men who seroconverted [41.5 $cm^2$, interquartile range (IQR) 31.0–54.5] compared with men who did not seroconvert (35.0 $cm^2$, IQR 26.30–45.0). The mean foreskin surface area was significantly higher among men who seroconverted [43.3 $cm^2$, standard error (SE) 2.1] compared with men who remained uninfected (36.8 $cm^2$, SE 0.5, $P = 0.001$). The mean foreskin surface area was smaller for younger men aged 15–24 years (35.0 $cm^2$, SE 0.8) than among older men aged 25–29 years (38.5 $cm^2$, SE 0.9, $P < 0.05$) and 30–49 years (38.4 $cm^2$, SE 0.8, $P < 0.05$.) Table 1 also shows the quartiles of foreskin surface areas.

As shown in Table 2, HIV incidence was 0.80/100 person-years among men with foreskin surface areas in the lowest quartile ( ≤26.3 $cm^2$), 0.92/100 person-years with foreskin surface areas in the second quartile (26.4–35.0 $cm^2$), 0.90/100 person-years with foreskins in the third quartile (35.0–46.0 $cm^2$) and 2.48/100 person-years in men with foreskin surfaces areas in the upper quartile (>44.6 $cm^2$) (chi-squared test for trend, $P < 0.01$.) HIV incidence was significantly higher among men with a foreskin surface area in the highest quartile compared with men with foreskin surface areas in the lowest quartile (unadjusted IRR 3.10, 95% CI 1.33–7.98). Compared with men aged 15–24 years, the unadjusted risk of HIV acquisition was significantly increased among men aged 25–29 years (IRR 5.03, 95% CI 1.98–15.27) and men aged 30 years or older (IRR 4.28, 95% CI 1.69–13.10). HIV risk was significantly lower among the better educated and non-Catholics, and the risk was increased in men reporting two or more sex partners in the past year. After adjustment for age, education, religion, condom use and number of sex partners in the past year, HIV incidence among men with foreskin surface areas in the highest quartile was significantly greater than that among men in the lowest quartile of surface area (adjusted IRR 2.37, 95% CI 1.05–5.31). This was lower than the unadjusted risk estimate (IRR 3.10), suggesting con-founding by the correlation between age and foreskin surface area. There was, however, no significant difference in HIV incidence between the lower three quartiles of foreskin surface area. Older age, lower education level and religion remained significantly associated with risk of HIV acquisition after adjustment (Table 2).

The cumulative HIV incidence among men enrolled in this study (1.27/100 person-years) was similar to the incidence of 1.33/100 person-years among control arm men who participated in the male circumcision trial [8], suggesting that the sample was representative of uncircumcised men in this rural population.

## Discussion

We found that the mean foreskin surface area among men who seroconverted to HIV was significantly larger than that among men who remained uninfected (Table 1), and that the risk of HIVacquisition was significantly increased among men with foreskins in the upper quartile of surface area compared with men in the lowest quartile of foreskin area (Table 2). We also observed a significant trend of increasing HIV incidence with an increasing quartile of foreskin area. These observations strongly suggest that larger foreskin size is a risk factor for HIV acquisition in uncircumcised men. There are no prior studies of the association between foreskin surface area and HIV acquisition, so our findings need to be replicated.

These findings, in addition to the observational studies and randomized trials, add plausibility to the hypothesis that the foreskin is a tissue vulnerable to HIV acquisition. The increase in risk of HIV acquisition among men with the largest foreskin surface areas may be due to the presence of a larger number of HIV target cells in the inner preputial mucosa that is exposed to infected vaginal fluids during sexual intercourse. It is also possible that men with larger foreskin surface areas may be more vulnerable to trauma of the foreskin mucosa during intercourse, increasing the risk of HIV acquisition.

Our findings may be of programmatic importance in that they suggest the need to minimize retention of residual foreskin tissue after male circumcision. This is particularly relevant to the forceps-guided procedure, which leaves 0.5–1.0 cm of mucosal skin proximal to the corona but less of a problem with the dorsal slit and sleeve procedures [17]. However, this is

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only a theoretical concern, as we did not observe a significant increased risk of HIV acquisition among men with smaller foreskin surface areas (Table 2), which are substantially larger than residual tissue retained after circumcision surgery.

The study has limitations. Only one measurement of foreskin surface area was made by each physician. Although the procedure for measuring surface area was standardized, and physicians were carefully trained, we did not do studies of repeated measurement to assess potential measurement error or studies of variation in measurements between observers. Additionally, the amputated foreskin is not necessarily rectangular, and variation in shape could lead to errors in calculating the surface area. Nevertheless, such errors, if they occurred, would not be differential between participants who did or did not acquire HIV infection, and thus systematic measurement errors are unlikely to account for our findings.

### Conclusion

A larger foreskin surface area was associated with an increased risk of HIV acquisition, which suggests that providers should avoid leaving excess residual foreskin tissue after circumcision.

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## Table 1

Median, interquartile range, mean, 95% confidence intervals for means and standard error for selected participant characteristics.

| Characteristic | *n* (%) | Surface area median (IQR) | Surface area mean (95% CI) | SE |
|---|---|---|---|---|
| All | 965 | 35.0 (26.3, 45.5) | 37.1 (36.2, 38.0) | 0.5 |
| HIV incidence status | | | | |
| Seronegative | 917 (95.0%) | 35.0 (26.0, 45.0) | 36.8 (35.8, 37.7) | 0.5 |
| Incident HIV | 48 (5.0%) | 41.5 (31.0, 54.5) | 43.3 (39.0, 47.6) | 2.1 |
| Age (years) | | | | |
| 15–24 | 380 (39.4%) | 32.0 (24.0, 42.6) | 35.0 (33.4, 36.5) | 0.8 |
| 25–30 | 269 (27.9%) | 35.4 (28.0, 48.0) | 38.5 (36.7, 40.4) | 0.9 |
| 30–49 | 316 (32.8%) | 36.0 (28.0, 47.0) | 38.4 (35.4, 37.6) | 0.8 |
| Surface area quartiles | | | | |
| ≤25th (7–26.3) | 252 (26.1%) | 21.4 (18.0, 24.0) | 20.9 (20.5, 21.5) | 0.3 |
| ≤50th and >25th (26.4–35.0) | 247 (25.6%) | 31.0 (28.3, 33.0) | 30.9 (30.5, 31.2) | 0.2 |
| ≤75th and >50th (35.2–45.5) | 225 (23.3%) | 40.0 (37.5, 42.0) | 40.0 (39.6, 40.4) | 0.2 |
| >75th (44.6–99.8) | 241 (25.0%) | 55.0 (50.0, 61.8) | 57.6 (56.3, 58.9) | 0.7 |

CI, confidence interval; IQR, interquartile range; SE, standard error.

## Table 2

HIV incidence rates, unadjusted and adjusted incidence rate ratios by quartile of foreskin surface area and participant characteristics.

| | *n* | No. of persons incident | Person-years | Incidence/100 person-years | Unadjusted IRR (95% CI) | Adjusted IRR (95% CI) | *P* |
|---|---|---|---|---|---|---|---|
| Quartile of foreskin surface area ($cm^2$) | | | | | | | |
| ≤25th (7–26.3) | 252 | 8 | 994.9 | 0.80 | 1.00 | 1.00 | |
| ≤50th and >25th (26.4–35.0) | 247 | 9 | 975.3 | 0.92 | 1.15 (0.39–3.42) | 0.82 (0.31, 2.19) | 0.69 |
| ≤75th and >50th (35.2–45.5) | 225 | 8 | 888.5 | 0.90 | 1.13 (0.37–3.42) | 0.87 (0.32, 2.32) | 0.77 |
| >75th (45.6–99.8) | 241 | 23 | 926.8 | 2.48 | 3.10 (1.33–7.98) | 2.37 (1.05, 5.31) | 0.04 |
| Age | | | | | | | |
| 15–24 | 380 | 6 | 1515.0 | 0.40 | 1.00 | 1.00 | |
| 25–30 | 269 | 21 | 1044.7 | 2.01 | 5.03 (1.98–15.37) | 4.16 (1.55, 11.19) | 0.01 |
| >30 | 316 | 21 | 1225.6 | 1.71 | 4.28 (1.69–13.10) | 4.00 (1.46, 10.74) | 0.01 |
| Education | | | | | | | |
| None/primary | 665 | 41 | 2589.9 | 1.58 | 1.00 | 1.00 | |
| Secondary/tertiary | 300 | 7 | 1195.4 | 0.59 | 0.37 (0.14–0.83) | 0.40 (0.18, 0.91) | 0.03 |
| Religion | | | | | | | |
| Catholics | 648 | 41 | 2530.3 | 1.62 | 1.00 | 1.00 | |
| Other religions | 317 | 7 | 1255.1 | 0.56 | 0.35 (0.13–0.77) | 0.37 (0.16, 0.82) | 0.01 |
| Condom use in past year | | | | | | | |
| Did not use condoms | 479 | 21 | 1874.5 | 1.12 | 1.00 | 1.00 | |
| Inconsistent | 335 | 23 | 1312.3 | 1.75 | 1.56 (0.83–2.97) | 1.02 (0.52, 1.98) | 0.96 |
| Used consistently | 133 | 4 | 526.6 | 0.76 | 0.68 (0.17–2.01) | 0.81 (0.27, 2.44) | 0.71 |
| Sex partners in past year for sexually active men | | | | | | | |
| One | 444 | 14 | 1755.7 | 0.78 | 1.00 | 1.00 | |
| Two or more | 419 | 33 | 1622.7 | 2.03 | 2.60 (1.33–5.16) | 2.36 (1.18, 4.72) | 0.02 |
| Tribe | | | | | | | |
| Muganda | 770 | 34 | 3028.9 | 1.12 | 1.00 | – | – |
| Other tribe | 195 | 14 | 756.5 | 1.85 | 1.65 (0.82–3.15) | – | – |

CI, confidence interval; IRR, incidence rate ratio.