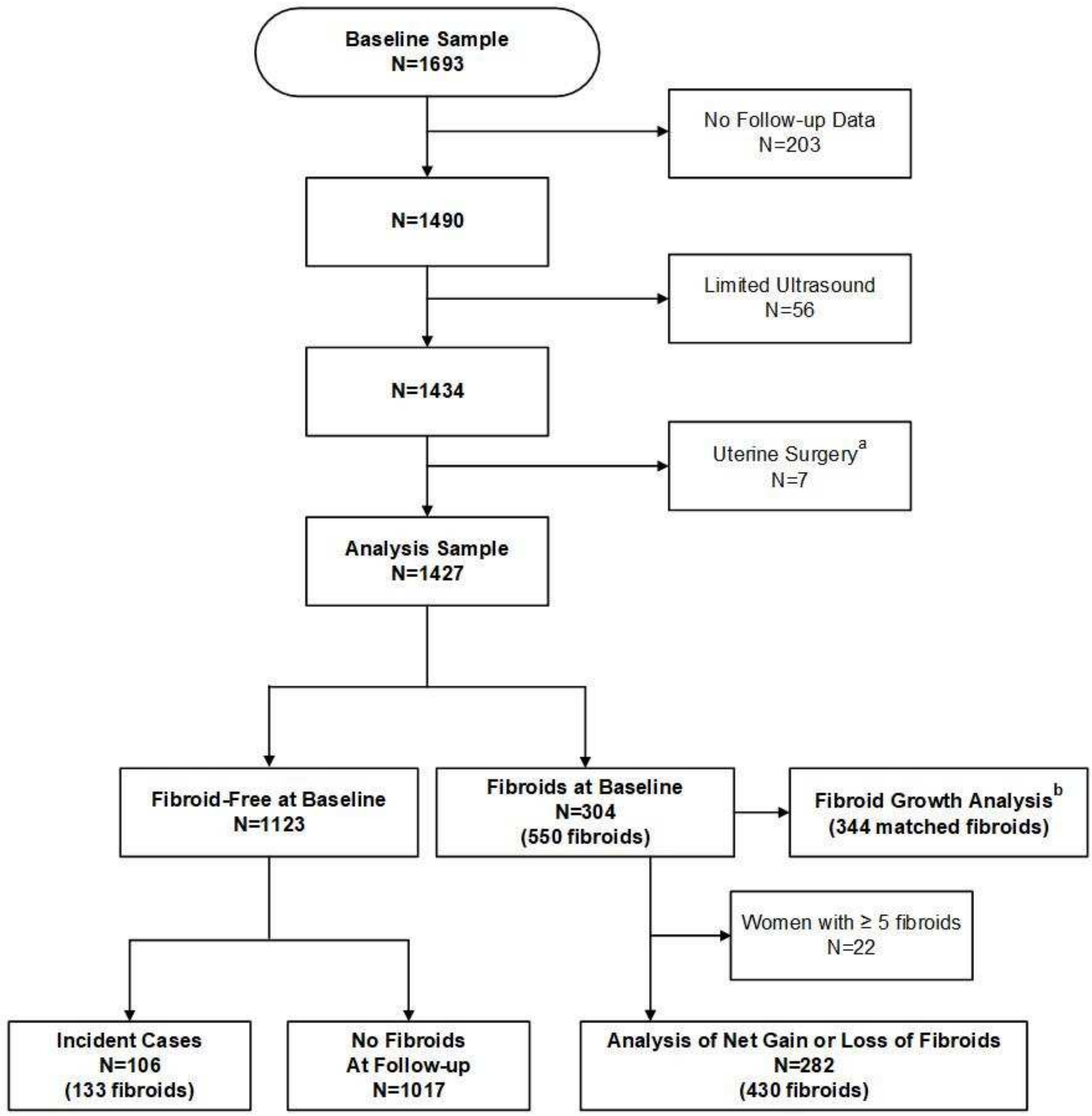
**Supplementary Materials** **Page**

### *1.1 Supplementary Methods: Flowchart of study sample*



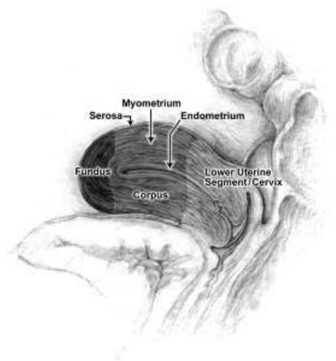

**Footnotes**

[a] Uterine surgeries included 3 women without baseline fibroids and 4 women with baseline fibroids. In addition, there was one woman with baseline fibroids excluded due to limited ultrasound who had uterine surgery during follow-up.

[b] The 344 matched fibroids for fibroid growth analysis were primarily identified from these 550 fibroids in this sample, but 9 of the matched fibroids were identified from 8 women whose ultrasound was limited, but adequate for matching and measuring those 9 fibroids.

### *1.2 Supplementary Methods: Ultrasound Assessment of Fibroids*

Ultrasound methods have been described in detail in Baird et al., 2015[1]. Critical details are reiterated here. Ultrasound examinations were conducted throughout the study with Phillips IU-22s, with the exception of one GE Logic 9 machine. The additional study training of sonographers included care in distinguishing fibroids from other pathologic changes in the uterus including adenomyosis and polyps. For each of the nine diameter measurements (three perpendicular diameters at each of the three separate assessments for each fibroid), caliper placement was from outer border to outer border. The fibroid location in the uterus was recorded similarly to Peddada et al.[2] Fibroid type was defined as "intramural" if it was mainly within the myometrium and did not impinge into the endometrial cavity. If a fibroid impinged upon the endometrial cavity, it was considered "submucosal." A fibroid was considered "subserosal" if it projected from the serosal (uterine) surface, distorting the uterine contour with one-third or more of its volume. Fibroid location was also determined with respect to the uterine axis, and divided into three categories (fundus, corpus, lower uterine segment/cervix) based on a reference picture kept at all sonography stations (shown below). In the primary text of this manuscript, the lower uterine segment/cervix is referred to simply as "lower uterine segment."



### *1.3 Supplementary Methods: Calculation of diameter equivalency of volume*

The diameter equivalency to volume cut-points for size groups were calculated by solving for an average diameter, D, using the volume formula for an ellipsoid (V =.52 x length x width x height = .52 x $D^3$. Solving for D, we get D = cube root of (V/.52). For example, a fibroid with volume of .52 $cm^3$ will have an average diameter of the cube root of 1 or 1 $cm^3$.

### *1.4 Supplementary Methods: Estimating lost fibroids in each fibroid-size category*

There were 58 women who lost fibroids in our analysis sample for net loss of fibroid (53 women lost 1 fibroid, 3 women lost 2 fibroids and 2 women lost 3 fibroids). The total net loss of fibroids was therefore 65 fibroids. That will be the basis for our estimates.

Extrapolating from the 35 fibroids in the 33 women who lost all their fibroids, we assume a loss rate for each fibroid-size category identical to what is seen for the 35 fibroids, i.e., 51%, 37%, 9%, 3%, 0% (Supplementary 2.1). Applying that loss rate, we estimated number of fibroids lost in each fibroid size category. Then, given the number in each size category among the 430 fibroids in the sample, we calculate percentage lost in each size category. The calculations for each size category are shown below.

| **Fibroid size, diameter equivalency** | **Proportion lost** | **Number lost** | **Total fibroids** | **Estimated percent lost** |
|---|---|---|---|---|
| <1 cm | 0.51 | .51x65=33 | 144 | 33/144=23% |
| 1-<2 cm | 0.37 | .37x65=24 | 173 | 24/173=14% |
| 2-<3 cm | 0.09 | .09x65=6 | 62 | 6/62=10% |
| 3-<4 cm | 0.03 | .03x65=2 | 22 | 2/22=9% |
| ≥4 cm | 0 | 0.0x65=0 | 29 | 0/29=0% |
| Total | | | | 65/430=15% |

***1.5 Supplementary Methods: Details of fibroid growth analysis***

We followed the growth analysis methods used by Peddada, *et al.*[2] Fibroid growth was examined in 344 fibroids identified as the same fibroid at baseline and follow-up. As in all prior studies of fibroid growth, lost fibroids could not be included because their follow-up volume could be anything from “0 $cm^3$” if the fibroid completely resolved, to just below our level of detection (~0.05 $cm^3$). This will result in overestimation of growth estimates and underestimation of shrinkage frequency. All fibroid growth was modelled based on a growth rate estimated by the change in the natural logarithm of volume (ln-volume) per 18 months, an interval close to our follow-up time for participants. Given the higher measurement variability among our triplicate measures of volume for small compared to large fibroids,[3] we estimated the residual variance in growth rate separately for each level of the 5-level fibroid-size variable. Woman characteristics (age category, number of baseline fibroids, uterine position of anteverted or retroverted) and fibroid characteristics (size, position in the uterus) were examined for associations with growth and in post-hoc analyses we examined interaction between age and fibroid size by including a cross-product term (age category x size category) as a fixed effect in the mixed model. For each fibroid, we estimated an average change in ln-volume per day as the ratio of the change in ln-volume to the elapsed time in days between ultrasound examinations. To express the model estimates over an 18-month time interval, similar to our follow-up time, we multiplied each “per day” ratio by 540 days. For ease of interpretation we converted the growth rate on the ln-volume scale to a percent change in volume per 18 months with the formula 100 x (exp(R) – 1) where R is the growth rate on the ln-volume scale. For example, if in 18 months the volume increased from a 2 cm average diameter fibroid (volume = 4.19 $cm^3$) to a 2.5 cm average diameter fibroid (volume = 8.12 $cm^3$) this would be an increase in ln-volume from ln(4.19) to ln(8.12), namely, 0.662. This is R. So, 100 x exp(0.662) – 1 =100 x 1.94 -1 = a 94% increase in growth/per 18 months. Outlier analyses in our fibroid growth model revealed 5 fibroids with residuals for growth >3 standard deviations from the mean, and we present results after removing those outliers from the analysis.

### *1.6 Supplementary Methods: Details of spline analysis of growth by fibroid size*

All 344 fibroids were included in the spline analysis. We developed a natural cubic spline mixed model with 4 knots (1 cm, 2 cm, 3 cm, 4 cm diameter equivalencies of volume), to regress change in ln-volume over 18 months (our measure of fibroid growth rate) on the natural logarithm (base e) of baseline fibroid size. The model included separate intercepts for each age category (4-level variable described earlier) so that the spline curves for the age categories were parallel. The knots were selected at the cut-points of our prior size categories. Therefore, each section between the knots had its own piecewise curve. For clinical relevance, those growth rates were then converted into percent change in fibroid volume for graphing.

We included 'woman' as a random effect to accommodate the correlation in growth rates for fibroids from the same woman. To accommodate possibly differential measurement accuracy for fibroids of different sizes, our model allowed different residual variances within our prior size categories.

### *1.7 Supplementary Methods: Estimating time for fibroids to grow from a given size to a larger size*

To calculate the average time elapsed as a fibroid grows from a volume $v_0$ to a volume $v_1$, we need an equation that relates elapsed time to the two specified volumes. We used our data to fit a regression model for change in ln-volume over 18 months as a natural cubic spline function (4 knots) of ln-volume with separate intercepts for each of 4 age categories. To derive an equation for elapsed time from our regression model for change in ln-volume, we regarded our fitted regression model as a differential equation and solved it.

To carry out this approach, we made two simplifying assumptions. First, we regarded the change in ln-volume per 18 months as an instantaneous derivative of ln-volume with respect to time, denoted $\frac{d}{dt} ln(v)$. Second, to enable a closed-form solution to the differential equation, instead of basing our calculation on the spline model used previously, we employed a less complex model where change in ln-volume per 18 months was modeled as linear in ln-volume but with separate intercepts for each age category. This simpler model fit almost as well as the more complex model; and, when graphed, the fitted trajectories appeared close.

Accordingly, suppose a fibroid has volume $v_0$ at time $t_0$ and grows to volume $v_1$ at time $t_1$. Given $v_0$ and $v_1$ (but neither $t_0$ nor $t_1$), we seek to calculate the elapsed time $t_1 - t_0$ for each age category. For a given age category, the differential equation that we need to solve is:

$$\frac{d}{dt} ln(v) = \alpha_A + \beta \cdot ln(v).$$

Here, $\alpha_A$ is the intercept for age category $A$ and $\beta$ is the slope. This first-order ordinary differential equation with variables separable is solvable by standard methods (Brauer and Nohel, 1967). The solution for an individual age category is:

$$t_1 - t_0 = \frac{1}{\beta} \cdot ln\left[\frac{\alpha_A + \beta \cdot ln(v_1)}{\alpha_A + \beta \cdot ln(v_0)}\right]$$

Because our regression model assessed time in units of 18 months, the elapsed time calculated from the solution is in those same units. An 18-month unit is 3/2 of a year. We report elapsed time in years.

The fitted regression model provides estimates of $\beta$, the 4 $\alpha_A$ parameters, and their variance-covariance matrix. Because the elapsed time is a non-linear function of the parameters, we used a Taylor-series approach (often called the delta method) to reduce bias and to estimate variance. We then used those point estimates to construct 95% Wald confidence limits.[4]

***2.1 Supplementary Results: Table 1 Characteristics of lost vs remaining fibroids***

**SUPPLEMENTARY TABLE 1**

**Baseline fibroid size and location for fibroids lost (from 33 women who lost all their fibroids) compared to baseline fibroid size and location of fibroids in 249 women who still had fibroids at follow-up**

| **Fibroid characteristic** | **Lost Fibroids[a]** N = 35 | | **Remainder of Baseline Fibroids[a]** N = 395 | | **P-value[a]** |
|---|---|---|---|---|---|
| | N | % | N | % | |
| Fibroid size ($cm^3$) at baseline | | | | | 0.05 |
| <0.52 [<1cm diameter][c] | 18 | 51 | 126 | 32 | |
| 0.52-<4.19 [1 cm – <2 cm] | 13 | 37 | 160 | 41 | |
| 4.19-<14.1 [2 cm – <3 cm] | 3 | 9 | 59 | 15 | |
| 14.1-<33.5 [3 cm – <4cm] | 1 | 3 | 21 | 5 | |
| ≥33.5 [≥4 cm] | 0 | 0 | 29 | 7 | |
| | | | | | |
| Fibroid position at baseline | | | | | 0.41 |
| Submucosal | 2 | 6 | 21 | 5 | |
| Intramural fundal | 10 | 29 | 92 | 23 | |
| Intramural corpus | 18 | 51 | 183 | 46 | |
| Intramural lower uterine segment | 3 | 9 | 11 | 3 | |
| Subserosal/pedunculated | 2 | 6 | 88 | 22 | |

[a]Analysis limited to baseline fibroids in the 282 women with 1-4 fibroids; lost fibroids are from women who lost all their fibroids and the remainder of baseline fibroids are from women who still had fibroids at follow-up;

[b]P-value from logistic regression with both fibroid size (ordinal variable) and position (class variable) in model.

[c]Diameter equivalency of the fibroid volume shown in brackets.

### *2.2 Supplementary Results: Table 2 Fibroid growth by characteristic, unadjusted*

**SUPPLEMENTARY TABLE 2**
**Unadjusted growth/18 mos by characteristic**

| Characteristic | Category | % Growth/18 mos | 95% CI |
|---|---|---|---|
| Age | 23-25 | 105 | 55, 171 |
| | 26-28 | 119 | 81, 163 |
| | 29-31 | 121 | 91, 156 |
| | 32-35 | 70 | 49, 95 |
| Fibroid size | < 1 cm | 185 | 147, 228 |
| | 1 cm - <2 cm | 95 | 73, 119 |
| | 2 cm - <3 cm | 62 | 37, 92 |
| | 3 cm - <4 cm | 21 | -6, 55 |
| | 4+ cm | 53 | 21, 94 |
| Number of fibroids | 1 | 91 | 70, 115 |
| | 2 | 119 | 81, 166 |
| | 3 | 107 | 64, 161 |
| | 4+ | 86 | 47, 136 |
| Uterine position | Anteverted | 100 | 82, 120 |
| | Retroverted | 94 | 59, 135 |
| Fibroid position | Submucosal | 146 | 81, 234 |
| | Intramural fundal | 121 | 88, 160 |
| | Intramural corpus | 103 | 81, 128 |
| | Intramural lower segment | 38 | -8, 107 |
| | Subserosal or penduculated | 70 | 46, 97 |



### *2.3 Supplementary Comment: Detailed review of prior studies of fibroid growth*

Of the eight prior studies, only two included more than 100 women, and those were retrospective studies based on medical chart review. Because the eligibility criteria for the prior studies differed, fibroid size distributions were also quite varied. Five of eight prior studies examined associations with baseline fibroid size, and three found faster growth of small fibroids,[5,6,7] as did we. The two studies that did not find an association had sampled mostly larger fibroids,[2, 8] consistent with findings that size was not strongly associated with growth in fibroids ≥2 cm in diameter.

Both Tsuda *et al.*[8] and Nieuwenhuis *et al.*[9] collected vascularity data and found increased growth with vascularity, but neither of these examined associations between fibroid size and growth. However, small size and greater vascularity have been linked in a study of pathology specimens.[10] These authors examined tissue from 20 fibroids collected during hysterectomies or myomectomies and divided them into four hypothesized developmental phases based on collagen matrix content: <3% collagen, 3%-15% collagen, 15%-50% collagen, >50% collagen. Fibroid size increased with matrix content, while vascularity and proliferation (PCNA staining) decreased with matrix content. Thus, it is likely that the larger fibroids in our study had more matrix and reduced vascularity, potentially accounting for their slower growth rates.



Fibroid shrinkage (≥20% shrinkage during the study's observation interval) was also noted in several other studies[2,5,6,11] and varied between 3% per 6 months to 26% per year. Our estimate of 11% over 18 months is within this range.

A problematic methodology for some of the prior growth studies was their apparent use of percent change in fibroid volume as their outcome measure in linear regression. This analytic approach is problematic because percent increase in volume can extend well beyond a 100%

increase, but percent decrease must be less than 100 percent. Given resulting non-normality of the outcome (growth) the confidence intervals and p-values regarding their growth data may be distorted.

***References***


1. Baird DD, Harmon QE, Upson K, et al. A Prospective, Ultrasound-Based Study to Evaluate Risk Factors for Uterine Fibroid Incidence and Growth: Methods and Results of Recruitment. Journal of women's health (2002) 2015;24:907-15.
2. Peddada SD, Laughlin SK, Miner K, et al. Growth of uterine leiomyomata among premenopausal black and white women. Proceedings of the National Academy of Sciences of the United States of America 2008;105:19887-92.
3. Moshesh M, Peddada SD, Cooper T, Baird D. Intraobserver variability in fibroid size measurements: estimated effects on assessing fibroid growth. Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine 2014;33:1217-24.
4. Brauer F, Nohel JA. *Ordinary differential equations : a first course.* New York: W.A. Benjamin; Number of pages.
5. Mavrelos D, Ben-Nagi J, Holland T, Hoo W, Naftalin J, Jurkovic D. The natural history of fibroids. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2010;35:238-42.
6. David M, Adams L, Stupin JH. Natural Size Development of Myomata - Ultrasound Observational Study of 55 Premenopausal Patients. Geburtshilfe und Frauenheilkunde 2014;74:75-80.

7. Armbrust R, Wernecke KD, Sehouli J, David M. The growth of uterine myomas in untreated women: influence factors and ultrasound monitoring. Archives of gynecology and obstetrics 2018;297:131-37.

8. Tsuda H, Kawabata M, Nakamoto O, Yamamoto K. Clinical predictors in the natural history of uterine leiomyoma: preliminary study. Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine 1998;17:17-20.

9. Nieuwenhuis LL, Keizer AL, Stoelinga B, et al. Fibroid vascularisation assessed with three-dimensional power Doppler ultrasound is a predictor for uterine fibroid growth: a prospective cohort study. BJOG : an international journal of obstetrics and gynaecology 2018;125:577-84.

10. Flake GP, Moore AB, Sutton D, et al. The natural history of uterine leiomyomas: light and electron microscopic studies of fibroid phases, interstitial ischemia, inanosis, and reclamation. Obstetrics and gynecology international 2013;2013:528376.

11. Ichimura T, Kawamura N, Ito F, et al. Correlation between the growth of uterine leiomyomata and estrogen and progesterone receptor content in needle biopsy specimens. Fertility and sterility 1998;70:967-71.