Supplementary Materials

Monetary Compensation

Compensation was given to the participants for transportation costs, time, and inconvenience associated with the study activities (Supplementary Table S1). Most compensation was linked to completion of the major activities associated with a clinic visit.

Fibroid Assessment

Sonographers conducting exams were registered diagnostic medical sonographers and had at least three years of gynecologic ultrasound experience. Study exams were conducted on Phillips IU-22s with the exception of one GE Logic 9 machine of a similar age. Magnification was performed as needed, but sonographers did not magnify to the point that the location position or type of fibroid could not be determined when reviewing images.

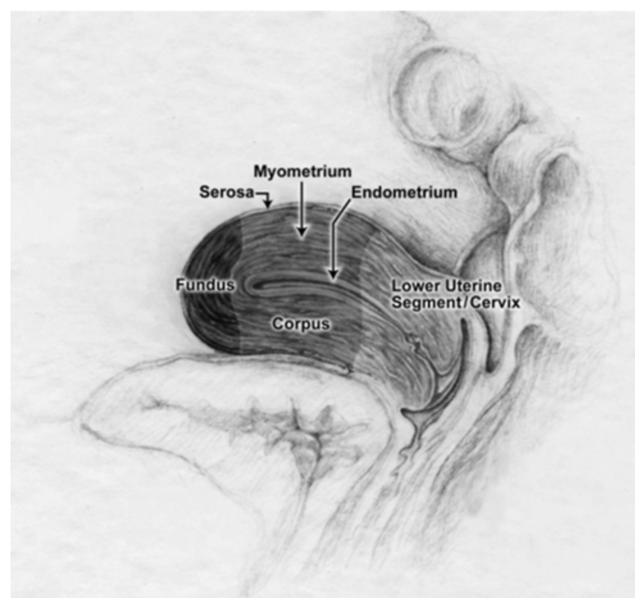
Additional training was conducted to assure a similar data collection approach by all sonographers. The training focused on detection, assessment, and recording of uterine fibroids. Fibroid identification was based on ultrasonographic criteria previously described in the literature, but extended to identify small fibroids (at least 0.5 cm in maximum diameter). Care to distinguish fibroids from other pathologic changes in the uterus was addressed by including training on differences between adenomyosis and fibroids and polyps and fibroids.

Examinations were not scheduled according to a specific time during the menstrual cycle. Ultrasounds were conducted transvaginally, and women were asked to void prior to examination. Transabdominal ultrasound examination was added if the transvaginal examination did not provide a complete assessment. If a fibroid-like echo pattern could not be visualized in all three planes, it was recorded as a ''questionable fibroid'' and the visualized diameters were recorded. The largest six fibroids were assessed in detail. The total number were counted, with truncation at 10 or more. Sonographers used a standardized data collection form that included a diagram of the uterus where each of the assessed fibroids was mapped and numbered.

The diameters of assessed fibroids were measured in three perpendicular planes (longitudinal, anterior-posterior, and transverse). Each fibroid was relocated and re-measured two additional times during the examination. Caliper placement for each diameter was from outer border to outer border. Therefore, a total of nine diameters were recorded for each fibroid.

Fibroid type (intramural, submucosal, subserosal, and pedunculated) and location (fundus, uterine corpus, and cervix/lower uterine segment) were assessed. 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 determined with respect to the uterine axis, and divided into three categories (fundus, corpus, cervix/lower uterine segment) based on a reference picture kept at all sonography stations (Fig. S1).

Still and video images were archived for each ultrasound examination and used for quality control. The head sonographer (TC) reviewed the first 10 ultrasound examinations with each study sonographer. Subsequently, for quality control, the head sonographer reviewed 8% of each sonographer's examinations each month (oversampled for those in which fibroids were detected), based on the archived images and the completed forms. Every sonographer had at least one examination reviewed each month, so the actual percent reviewed was 29.5% ($n = 501$). Of these ultrasound examinations, one or more fibroids had been detected in 231 of the examinations, while no fibroids had been detected in the remaining 270 of the examinations. After completing the review process, the head sonographer identified a total of six distinct and one questionable fibroid that had not been detected in the initial examination. These were added to the data set. Four of these additions were for women who had no fibroids detected during the examination; the other three were for women who had other fibroids detected during the examination. Thus, an estimated 1.5% of the women considered fibroid free at enrollment (baseline) may actually have fibroids. The head sonographer also found one recorded fibroid that she determined to be a hemorrhagic cyst of the ovary, and it was removed from the data set. Thus, an estimated 0.4% of women with fibroids may actually be fibroid free.



SUPPLEMENTARY FIG. S1. Fibroid location was determined with respect to the uterine axis, and divided into three categories (fundus, corpus, cervix/ lower uterine segment) based on a reference picture kept at all sonography stations.

Table S1. Monetary Compensation for Study Participants

All activities required for enrollment: $150
Return of the Early Life questionnaire: $30
Menstrual diary after enrollment visit: $20
Follow-up visit 1 activities, including menstrual diary: $160
Follow-up visit 2 activities, including menstrual diary: $160
Follow-up visit 3 activities, including menstrual diary: $160
Bonus if all visit activities have been completed: $100
Activities offered to only a subset of the participants
Six-month blood draw: $35
Time-outside diary: $25

Clinic Measures

The procedures for taking the clinic measurements are described in Table S2.

Processing and Storage of Biological Specimens

Initial specimen processing was done at the Henry Ford Health System laboratory, Molecular Epidemiology and Research Laboratory (MERL). Processed urine and blood aliquots as well as vaginal swabs were boxed and stored in –20C or –80C. Approximately every 2 weeks, full boxes, separated by material type, were shipped on cold packs or dry ice respectively to Social and Scientific Systems (SSS), the contract company that provides support for the Study of Environment, Lifestyle, and Fibroids in North Carolina. There further processing was done with the serum, plasma and urine. Table S3 summarizes the biospecimen collection and storage at baseline.

Red-top tubes were allowed to clot. The labeled samples were transported in coolers on cold packs to MERL in midtown Detroit. Blood processed for serum and plasma were centrifuged at +4C. From the red-top tubes, MERL creates a clot aliquot (Sarstedt 10-mL capacity, polypropylene vial No. 60.551.001) and a serum aliquot (Sarstedt 8-mL Polypropylene vial No. 60.542.080). Upon arrival at SSS the clots are relabeled for final storage and the serum is thawed at room temperature for 1 hour. Both serum samples from the same ID are pooled together, then aliquotted into 1-mL aliquots (1-mL Nalgene vial No. 5000-0010). For plasma, MERL processes the 10-mL ethylenediaminetetraacetic acid (EDTA) tube into a 5-mL packed cell volume (PCV) and 5-mL plasma aliquot (Nalgene 5-mL vial No. 5000-0050). Upon arrival at SSS the PCVs are relabeled and the plasma is thawed at room temperature for 1 hour, then aliquotted into two 1-mL aliquots (1-mL Nalgene vial No. 5000-0010) and five 0.4-mL CryoBioSystem™ straws. For urine, MERL processes, the specimen into one 50-mL vial (Sarstedt 50mL No. 62.559) and two 5-mL aliquots with 8% Glycerol (Nalgene 5-mL vial No. 5000-0050). The time between blood and urine collection and completion of processing at MERL varied from less than 1 hour to nearly 24 hours because on rare occasions when enrollment visits occurred late in the day, the laboratory processing was done the following day (99% of samples were processed the same day). The median time for blood was 3 hours with 0.5% at 8 hours or longer. The median time for urine was 8 hours (because women collected first morning urine at home on the day of their enrollment clinic visit), and 23.1% were 12 hours or greater. Upon arrival at SSS the 5-mL urine/glycerol aliquots are relabeled and the 50-mL urine (without glycerol) aliquot is thawed at room temperature for 1 hour, after which a dipstick urinalysis is performed (Multistix Pro 100 on a Clinitek 500 urinalysis analyzer). Then the urine is aliquotted into four 5-mL aliquots (Nalgene 5-mL vial No. 5000-0050) and twenty 0.4-mL CryoBioSystem™ straws. The urinalysis measures are listed in Table S4.

Table S2. Procedures for Taking the Clinic Measures

Measurement	*Procedure*	*Equipment*
Height	Standing height (in feet and inches) was measured twice and recorded to the nearest ¼ inch. If the two heights differed by greater than ½ inch, the measurement was repeated and recorded a third time.	Perspective Enterprises AIM-101 stadiometer
Weight	Weight (in pounds) was measured twice and recorded to the nearest 1/10 pound. If the two weights differed by greater than 1 pound, the measurement was repeated and recorded a third time.	Tanita BWB 800 electronic scale
Skin reflectance	Skin reflectance was measured (and recorded) three times using the upper inner right arm. In the case of tattoos, bruises, scars, or birthmarks, the upper inner left arm, or an area of the back not normally exposed to sun (in that order) could be used as alternative measurement locations.	Cortex Technology DSM II ColorMeter
Blood pressure and pulse	After sitting quietly for 5 minutes, the participant's systolic and diastolic blood pressure and pulse rate were measured twice using an automatic monitor. The measurements were taken using the right arm (or left arm if right arm was contraindicated).	Omron Intellisense automatic blood pressure monitor

Table S3. Processing and Storage of Biological Specimens (Blood, Urine, Vaginal Swabs)

Specimen	*Collection container*	*Volume collected*	*Aliquots created at MERL*	*Aliquots created at SSS*	*Final temp.*	*Additive/ preservative*	*Potential analytes*
Blood	1 Royal blue Vacutainer[a]	6 mL	Not aliquotted	None	–20C	K_2EDTA	Metals
Blood	2 Red top Vacutainers	10 mL each	5-mL serum and 5-mL clot	Serum pooled; 1-mL aliquots created	–80C	None	Vitamin D, serology, DNA
Blood	1 Lavender Vacutainer	10 mL	Plasma & PCV	Two 1-mL plasma aliquots and five 0.4-mL plasma straws	–80C	K_2EDTA	To be determined, DNA (PCV)
Blood	1 Yellow top Vacutainer	6 mL	Not aliquotted	None	–80C	ACD-B	DNA
Blood	1 Green top Vacutainer	10 mL	2.5 ml processed for immunologic analyses (5 tubes); remainder for 1-ml plasma aliquots at HFHS		–80C	Sodium heparin	Regulatory T-cells, cytokines
Blood	1 Lavender Vacutainer	2 mL	Delivered to HFHS hospital clinical laboratory			K_2EDTA	Hemoglobin
Urine	Collection cup	90-mL capacity	One 50-mL vial, two 5-mL aliquots with 8% glycerol	Four 5-mL aliquots (Nalgene 5-mL vials, #5000-0050), twenty 0.4-mL straws CryoBioSystem™	–80C	2 Aliquots with 8% glycerol; remainder without glycerol	Hormones, STDs (antigens), toxicants (e.g., BPA, phthalates
Vaginal swab	Transport tube (medium)	1 swab	No processing		–80C	Preservative media, Aptima™	PCR for STDs
Vaginal swab	Transport tube (dry)	1 swab	No processing		–80C	Stored dry in sleeve, Epicentre™	Bacterial vaginosis

[a]BD Vacutainer®.

ACD-B, adenosine citrate dextrose, solution B; BPA, bisphenol A; HFHS, Henry Ford Health System; MERL, Molecular Epidemiology and Research Laboratory; PCR, polymerase chain reaction; PCV, packed cell volume; SSS, Social and Scientific Systems (Durham, NC); STD, sexually transmitted disease

Table S4. Urinalysis Measures

Urine Factor	*Range*	*Units*	*Comments*
Clarity	Clear, cloudy		Read visually by technician
Color	Light yellow, yellow, brown, red, green, etc.		
Glucose	Negative, 100, 250, 500, 1000, 2000 or more	mg/dL	
Ketones	Negative, trace, small, moderate, large		
Specific gravity	1.000, 1.005, 1.010, 1.015, 1.020, 1.025, 1.030		
Blood	Negative, trace, moderate (nonhemolyzed) trace, small, moderate, large (hemolyzed)		
pH	5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5		
Nitrites	Negative, positive		
Leukocyte esterase	Negative, trace small, moderate, large		(Indication of white cells)
Protein	Negative, low, 1+, 2+, 3+, 4+		Alternative range = negative, 15, 30, 100, 300, 2000 or more (mg/dL)
Creatinine	10, 50, 100, 200, 300	mg/dL	
P:C ratio	Normal, normal dilute, abnormal[a]		Protein:creatinine ratio

[a]Normal dilute is recorded if protein is negative and creatinine = 10; abnormal is recorded if protein is 15 and creatinine is 100 or less or protein is 30 and creatinine is 200 or less or protein is 100 or greater regardless of the creatinine concentration.

Table S5. Prevalence of Fibroids by Age of Participant

Age	*Fibroid prevalence n (%)*
23–25	36 (10)
26–28	87 (21)
29–31	115 (25)
32–35[a]	140 (32)
Total	378 (22)

[a]No one over 34 years old was recruited, but some 34-year-olds had turned 35 by the time they had their ultrasound examination.