Supporting Information Manuscript for

Lifetime reproductive success of high-altitude ethnic Tibetan women: oxygen delivery phenotypes and genotypes

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Materials and Methods

This Supporting Information (SI) provides a detailed description of the materials and methods used and the analyses of the number of pregnancies.

Study population

The study population consists of Nepali citizens who self-identify as ethnically Tibetan: they speak Tibetan dialects and share economic, religious, and sociocultural practices common among highland Tibetans (Cho et al., 2017). Villages range in altitude from 3500 to 4100 m (11,550–13,530').

Study sample

We invited 430 women who participated in our 2012 study in Upper Mustang District, Nepal, to join a follow-up study in 2019. The 2012 study recruited women 39 years and older who had been married or pregnant and were native residents of Upper Mustang, as well as another sampling location in Nepal (1). We have reported on this larger sample of 1,008 women (1-4). The criterion for the present study was Upper Mustang residence and participation in 2012. Identification confirmation involved matching a woman's name, residence village, animal year of birth, husband's name (if applicable) in 2012, and the names and animal birth years of the oldest and youngest children. Upon confirming eligibility, research assistants obtained written informed consent and interviewed each woman in the local Tibetan dialect, using established methods (2). The 417 who provided an interview and biological data in 2012 and 2019 form the sample for this report.

Data collection

Data collection centered on temporary laboratories established sequentially in the three largest area villages: Lo Monthang (3800 m (Barigo E7, BARIGO Barometerfabrik GmbH, Baden-Württemberg, Germany; latitude, longitude 29º10'N, 83º57'E, Garmin eTrex HC series, Garmin International Inc., Olathe, KS), Tsarang (3500 m; latitude, longitude 29º5'N, 83º56'E), and Ghami (3500 m; latitude, longitude 29º3'N, 89º53'E). The research team included U.S.-based researchers, ethnic Tibetan female nurses, and research assistants, including a community member from each village with a temporary laboratory.

Data collection took place in buildings without climate control. During data collection, indoor and outdoor temperature, barometric pressure, and relative humidity varied with altitude and weather (Beall et al., 2021). The barometric pressure between 6 a.m. and 7 a.m. averaged 481, 494, and 497 mmHg at Lo Monthang, Tsarang, and Ghami, respectively. Simultaneously, the outdoor temperature averaged 13.3 (56 ºF), 16.1 (61 ºF), and 18.9 ºC (66 ºF), respectively; the relative humidity averaged 61, 58, and 48%, respectively.

Biological measurements included height in centimeters without shoes, weight in kilograms corrected for clothing, calculated body mass index (BMI, kg/m^2) and body surface area (BSA, m^2). Percentage of oxygen saturation of hemoglobin, hemoglobin concentration (HB), and pulse were measured noninvasively by finger plethysmography (Masimo Pronto 7®, Irvine, CA) with a described protocol (1, 5). The average of three seated resting blood pressure measurements was used to evaluate systemic pressures. The best of three efforts at providing forced expiratory volumes at 1 and 6 seconds evaluated pulmonary function. (6). We report Western ages (7).

Echocardiogram protocol

An experienced echosonographer performed echocardiographic (echo) studies to measure cardiopulmonary blood flow and anatomy, using an Acuson Cypress ultrasonograph (Siemens Medical Solutions, Erlangen, Germany). Studies were evaluated blindly as to the source of individual recordings. The echosonographer obtained standard parasternal, apical, and subcostal two-dimensional views. We acquired color flow-directed pulsed-wave Doppler measurements of transvalvular flows and continuous-wave Doppler measurements of the tricuspid regurgitant flow gradient and the velocity-time integral of right ventricular outflow to the lungs (RVOTvti) to estimate pulmonary artery systolic pressure and pulmonary blood flow, respectively (8, 9). The interior diameter at the base of the right ventricle at end-diastole and the left atrial diameter at end-systole were used as echo estimators of right ventricular and left atrial sizes. Left ventricular (LV) fractional shortening (LV FS) was calculated as the LV end-diastolic dimension (LVEDD) minus the LV end-systolic dimension divided by the LVEDD. Chamber dimensions were indexed by BSA to control for body size, whereas pressure and flow velocity are independent of body size (Liu and Yin, 1987). The early (E) and late (A) diastolic transmitral velocities were recorded from pulsed wave Doppler. Their ratio (E/A) provided an echo estimator of LV diastolic function. Tissue Doppler echo measured the ratio between early mitral inflow velocity and mitral annular early diastolic velocity (E/e') that was used as a surrogate of left ventricular filling pressure and diastolic function. Cardiac output was measured as the LVOT diameter x its velocity time integral x heart rate and indexed for BSA. The ultrasonograph recorded a single-lead electrocardiogram. Measures obtained with this noninvasive technique correlate strongly with those obtained with cardiac catheterization (Allemann et al., 2000 (10)), an invasive technique inappropriate for a field study of healthy individuals in a rural, out-of-hospital setting. Using chamber dimensions rather than volumes may introduce errors. Yet, epidemiological studies commonly use dimensions, as error is minimal without chamber remodeling. Similarly, Doppler echo-derived pressures and flows are surrogates (8). However, using these surrogates is necessary for this type of fieldwork. Three hundred ninety-seven women had acceptable echo examinations.

Hypoxic ventilatory and hypoxic heart rate response protocol

As a dynamic physiological challenge to oxygen delivery, we acquired one to three measurements of hypoxic ventilatory response (HVR) and hypoxic heart rate response (HHRR). Seated women breathed through a mouthpiece while using a nose clip. The mouthpiece connected to a one-way rebreathing circuit with a breathing valve (2700 Series, Large, Hans Rudolph). We recorded measurements while the woman breathed room air through the open circuit for 2–3 minutes, followed by a hypoxic stimulus (3–5 min) induced by rebreathing through the closed circuit long enough to produce a 10% decrease from baseline oxygen saturation. A pulse oximeter placed on the forehead with a headband

(Radical-7 Masimo Corp., Irvine, CA) continuously monitored the percentage of oxygen saturation of hemoglobin and pulse.

The change in ventilation (L) per percentage decrease in SpO2 (ΔV/ΔSpO2) yielded the ventilatory response to hypoxia (HVR). The change in heart rate per percentage decrease in SpO2 (ΔHR /ΔSpO2) during the same HVR maneuver provided the hypoxia heart rate response (HHRR). The reported measurements are the averages of the variables' values during a minimum of 30 seconds of breathing room air at baseline and hypoxic air in the circuit.

Thirteen women were ineligible based on signs of heart disease detected during the echo, or a diagnosis or symptoms of tuberculosis (4), goiter (1), or cancer. (1) Three hundred seventy-six women were eligible for a test of resting ventilation and heart rate and the response to additional hypoxia. The investigators stopped 10 studies for technical reasons; five women stopped their studies. Three-hundred sixty-four women had technically acceptable HVR and HHRR test results.

Data analyses

Modeling the relationship between physiological and reproductive variables

A novel statistical approach permitted flexible modeling to detect biologically meaningful relationships of these multiple traits with reproductive success, measured as the number of pregnancies and livebirths (Supporting Figure 1). We analyzed three sets of women: those with complete data (n=364), the full sample (n=417, some of whom did not provide an HVR test or echo), and a subset of women who were missing the HVR test or echo (n=53).

Simple Exploratory Data Analysis. Data processing guided by exploratory data analysis is a crucial first step for any data analysis or modeling. A simple sample exploration showed 115 potential covariates, candidate outliers, and blocks of missing values. The candidate outliers and missing values were checked and corrected if necessary.

Variable Reduction. To isolate influential physiological features among multiple possibilities in effectively predicting two outcomes (pregnancies and livebirths), we performed variable reduction of 115 potential covariates/variables, using two approaches: random forest (RF) and tree-based sure independence screening (SIS) With the RF approach, we searched for variables of importance collectively on all variables. With the tree-based sure screening procedure, we evaluated the predictability of each of the 115 variables, one at a time, based on the residual mean square errors of tree-based modeling (Tree) for each outcome by each covariate. RF and Tree models handle missing values easily and are nonparametric models without assuming the shapes of relationships of the covariates with an outcome. The single Tree analyses may be considered a generalization of the original SIS (11) to provide a quick, flexible, non-model-based screening of the features. Combining the results of RF and Tree screening with the support of domain knowledge, we reduced the number of covariates to 50 in our investigation of important predictors (from the covariates) below. Supporting Table 2 below describes the predictors.

Three Group Analyses. Because our sample contains a subgroup that did not have echo or HVR data, our comprehensive modeling of lifetime reproductive successes using these 50 variables involved three data sets: 1) the ***full sample*** *of 417 women*, 2) the **complete** *sample of 364* women with completed echo and HVR data, and 3) the ***small subset*** *of 53 women* who did not have echo and HVR data. Supporting Table 1 below describes the three groups. The right-hand column shows that the subset of women without echo/HVR was a mean of 7 years older than the others, had longer marriages, weighed less, had a lower percentage of oxygen saturation measured at the fingertip and the forehead, and had two or more miscarriages. These differences support the decision to analyze the third group separately.

Comprehensive Modeling. We used *parametric and nonparametric statistical modeling* to obtain biologically meaningful and interpretable models and probe influential predictors of lifetime reproductive success from these 50 candidate covariates. Two types of models analyzed multiple traits simultaneously: 1) interpretable mixed linear-nonlinear models for the sample with complete echo/HVR data (complete sample), and 2) tree-based models for all three samples, as shown in Supporting Figure 1 below.

\ Supporting Figure 1 below. Summary of data analysis steps.

A. Mixed Linear and Nonlinear Models. We developed our final interpretable parametric models for each outcome by using the *full sample* and the *complete data sample* with echo and HVR data, via the following five steps.

First, we performed further *exploratory data analysis*, using scatterplots of the outcomes (pregnancies and livebirths) with the 50 potential covariates, superimposed with a nonparametric smoothing curve called a Loess curve, *to examine possible relationships of the covariates with each of the outcomes.* As shown in Supporting Figure 2 below, the relationships of three covariates, age, BMI, and HB, do not look linear. The women 63 and older had about the same number of pregnancies. In contrast, successively younger women had fewer pregnancies, roughly consistent with the history of family planning, which became available to women in Nepal in the 1990s, and the subsequent decline in fertility (2).

\ Supporting Figure 2 below. Exploratory plots of the relationship between the number of pregnancies and livebirths with age, BMI, and hemoglobin.

Second, we performed a *hybrid, semi-parametric model analysis* by using generalized additive modeling (GAM) of the outcomes with the 50 covariates. Age, BMI, and HB were entered in the GAM as potential predictors via unspecified smooth curves (hence nonparametric), while the other covariates were entered via linear parametric relationships. The shape of estimated nonparametric curves from our GAM analysis (GAM1) confirmed the nonlinear relationships in the first step, demonstrating the need to transform age, BMI, and HB before using them as potential predictors in Poisson regression modeling.

Third, we determined the *specific transformations* for age, BMI, and HB. We fitted change point models by using R package vrcp (Ganocy & Sun, 2015) to determine a two-segment relationship between the number of pregnancies with age and another with hemoglobin mimicking a slight bulge at intermediate values for HB. In addition, we fitted the best quadratic model for the relationship between the number of pregnancies and BMI. These nonlinear relations are biologically sensible, as ever-increasing HB or BMI would not occur in a population, and births stop at menopause. These nonlinear reactions denoted as $f_{age}(x)$, $f_{hb}(x)$, and $f_{bmi}(x)$ provided the transformed variables: age1=$f_{age}(age)$, HB1=$f_{hb}(hb)$, BMI=$f_{bmi}(BMI)$. Transformed variables age2, hb2, and BMI2 were determined similarly for the number of livebirths.

\ Supporting Table 3 below.

Fourth, we fitted a *Poisson regression via generalized linear modeling (GLM) to a combination of the original and the transformed variables* for age, BMI, and HB. The resulting models are <u>*mixed linear-nonlinear parametric models*</u>. To check the adequacy of these mixed models in the multivariate setting, we fitted three competitive models for the number of pregnancies (*y*):

$$model\ 1: GLM\ (\ y \sim age1 + BMI1 + hb1 + other\ linear\ \&\ interaction\ terms |\ Poisson)$$

$$model\ 2: GLM(\ y \sim age1 + BMI + hb + other\ \ linear\ \&\ interaction\ terms |\ Poisson)$$

$$\begin{aligned} model\ 3: GAM\ (y \sim s(age) + s(BMI) + s(hb) \\ + other\ linear\ \&\ interaction\ terms |\ Poisson\ ) \end{aligned}$$

The first two are Poisson regression models of the number of pregnancies by using observations from women with complete echo and HVR data and incorporating the three transformed variables and other covariates in the original scales. Model 1 transforms all three covariates, age, BMI and HB, while Model 2 transforms only the most influential predictor, age, in the multivariate setting. Model 3 is a GAM that fully models age, BMI, and HB as nonparametric curves by using smoothing *splines* in the multivariate setting.

This third model serves as the most flexible benchmark to assess the adequacy of Models 1 and 2.

For the number of livebirths, we fitted the following three competitive models:

$$model\ 1': GLM\ (\ y \sim age2 + hb2 + other\ linear\ \&\ interaction\ terms;\ Poisson)$$

$$model\ 2': GLM(\ y \sim age2 + hb + other\ \ linear\ \&\ interaction\ terms;\ Poisson)$$

$$model\ 3': GAM\ (y \sim s(age) + s(BMI) + s(hb) + other\ linear\ \&\ interaction\ terms, Poisson\ )$$

For predicting the number of pregnancies, the nonlinear relationships for age, BMI, and HB are as follows:

(1)

$$\begin{aligned} f_{hb}(x) &= \begin{cases} -0.242 + 0.149x, & x \leq 13.67 \\ 2.017 - 0.016x, & x > 13.67 \end{cases} \\ f_{BMI}(x) &= (x - 22.52)^2 \\ f_{age}(x) &= \begin{cases} 0.056 + 0.029x, & x \leq 63 \\ 1.914, & x > 63 \end{cases} \end{aligned}$$

This equation (1) leads to the transformations $age1 = f_{age}(age), BMI1 = f_{BMI}(BMI)$, and $hb1 = f_{hb}(hb)$.

For predicting the number of livebirths (y), the nonlinear relationships are

(2)

$$\begin{aligned} g_{hb}(x) &= \begin{cases} -0.389 + 0.161x, & x \leq 13.4 \\ 1.942 - 0.012x, & x > 13.4 \end{cases} \\ g_{BMI}(x) &= (x - 21.95)^2 \\ g_{age}(x) &= \begin{cases} 0.050 + 0.0294x, & x \leq 63 \\ 1.901, & x > 63 \end{cases} \end{aligned}$$

This equation (2) leads to the transformations $age1 = g_{age}(age), BMI1 = g_{BMI}(BMI)$, and $hb1 = g_{hb}(hb)$.

Fifth, we performed *100 repeated 10-fold cross-validations (CVs)* to examine the performance of the three competing models for each outcome. Specifically, for each 10-fold CV, we randomly partitioned the data into 10 sub-datasets of approximately equal size and used each as a test set. At the same time, the remaining nine subsets formed a training set to derive three estimated models. In each case, we computed the mean residual square error (MRSE) of the test set, leading to 10 MRSEs for a 10-fold CV for each of the three comparative models. Next, we calculated the mean and variance of the 10 MRSEs and denoted them as mMRSE and vMRSE. Since a random partition may not lead to 10 similar subsets, we randomly repeated the 10-fold CVs 100 times, leading to 100 mMRSEs and 100 vMRSEs. We then calculated performance measures: m1=mean of 100 mMRSEs, m2=variance of 100 mMRSEs, m3=mean of 100 vMRSEs, and m4= variance of 100 vMRSEs. Small values of m1-m4 indicate good performance. The best performer in consideration of m1-m4 collectively was our choice for the final model, Model 1'.

$$model\ 1': Poisson\ (\#livbirth \sim age2 + hbavg2 + other\ linear\ and\ interactive\ terms),$$

The results for models 1-3 and 1'-3' based on m1-m4 are shown in Supporting Table 4 below.

B. Tree-based Models. We performed a *nonparametric analysis* of the three data sets by using tree-based models: one for the *complete* data *sample* with echo and HVR (364 observations), one for the *full sample* (417 observations) and another for the *small subset* of 53 observations of those without either echo or HVR measurements. The tree-based models can efficiently incorporate missing information and nonlinear complex relationships. Thus, the tree-based regression analysis (classification and regression tree) used all available data to identify the *combinations* of characteristics of women at the extremes of reproductive success measured as the number of pregnancies and livebirths.

Comparisons of Parametric Models and Tree-based Models. We compared the *important variables* selected by the GLM of the outcome by the mixture of the original and transformed variables and the tree-based models for the complete sample (364 observations). We also compared the three tree-based models for the full, complete data sample, and *small subset* samples. *The carefully chosen parametric models and tree-based models complement each other.* The parametric analysis yields statistical significance, while the tree-based models do not indicate statistical significance. Parametric models can be used to assess the strength and relative importance of independent variables for outcome measures. The tree-based analyses handle missing values, identify variables of importance for each outcome, and corroborate the findings from the parametric modeling by using GLM.

Genomic Analyses. For all genomic analyses, we used the same genome-wide genotype data described in (3) and available at https://datadryad.org/stash/dataset/doi:10.5061/dryad.bp46m. The data were phased and imputed as described in (3), resulting in genotype data for approximately 3.5 M single nucleotide polymorphisms (SNPs) for these women. We used these data to conduct a genome-wide association study (GWAS) and candidate SNP analysis of the independent variables included in our final models, except for the reproductive variables (i.e., mother's age at 1st birth and number of miscarriages) for which GWAS results are reported in (3). Our analyses include SNPs with minor allele frequency of ≥ 5%. For each variable, we performed stepwise regression by using the covariates age, age^2, altitude of test, relative wealth, menopausal status, InspO2RoomAir, perfusion, and fingertip temperature, and we chose the regression model with lowest Akaike information criterion (AIC) values. The covariates selected for each variable subjected to GWAS are as follows.

- AVGRFRoomair: age
- BMI: Age+RelativeWealth
- Dias: altitude
- HB: InspO2RoomAir+fingertip temperature
- HHRR: age + Menopausal status
- LADIndex: age^2
- Laterale: age^2 + age + Menopausal status
- LVOTDiamIndex: age

- Pulse: altitude
- RVOTvti: InspO2RoomAir + altitude +fingertip perfusion
- Sat (fingertip): age + fingertip perfusion
- Sat (forehead): age + fingertip perfusion

GWAS scan the entire genome to identify SNPs where a genotype associates with a phenotype of interest. To avoid spurious association results, we conducted an additional GWAS in which we collapsed the heterozygotes and the minor allele homozygotes into a single genotypic category and restricted our attention to SNPs where we observed at least 30 individuals in the collapsed genotype class. We retained the genome-wide significant signals from the 3-genotype GWAS only if they remained significant after collapsing the heterozygotes and minor allele homozygotes into a single class. As in (3), GWAS was performed by using GEMMA v0.94.1 (12). A univariate linear mixed model (LMM), as implemented in GEMMA (https://github.com/genetics-statistics/GEMMA), controlled for both population structure and genetic relatedness. All women had information on the phenotypes for the focal traits. The standardized genetic covariance matrix was calculated from this data set and used for LMM. Likelihood ratio test (LRT) p-values from GEMMA assessed the significance of genetic associations. An association was accepted as genome-wide significant if the p-value was equal to or lower than 5x10-8.

Candidate SNP analyses tested the narrow hypotheses that the *EGLN1* and *EPAS1* SNPs with the strongest selection signals measured by using the population branch statistic were associated with a phenotype in our final models. For *EGLN1*, we chose rs186996510, the nonsynonymous sequence variant D4E (aspartic acid changed to glutamic acid) (13). For *EPAS1*, we chose from the long haplotype the SNP with the highest population branch statistic: rs374487821. We report the associations without Bonferroni correction because we are testing one SNP.

Supporting Analyses.

Pregnancies

The parametric analysis showed that women 63 years and older, who reported no access to family planning technology (2), had the most pregnancies (Supporting Table 5 below). An earlier age at first birth and a longer marriage predicted more pregnancies; both reflect longer exposure to the risk of pregnancy. A higher percentage of oxygen saturation of hemoglobin (forehead reflectance measurement), HB of approximately 13.7 gm/dL, and lower HHRR predicted more pregnancies.

\ Supporting Table 5 below. General linear model analysis of the number of pregnancies among the 364 women with complete response information (Quasi $R^2 = 0.50$).

The nonparametric, qualitative, tree-based analysis (Supporting Figure 6 below) identified length of marriage as the main factor distinguishing women with few or many pregnancies (lowest or highest deciles). Reading from the top of the tree labeled "length of first marriage" and down the left side shows that a marriage of 8 years or less describes women averaging just 2.3 pregnancies. The right-most branch of the tree describes the 80% of the sample with a long marriage and a first birth before the age of 27. Two sets of women in this group had more than nine pregnancies. The sets shared left atrial diameters normalized for BSA (LADIndex) at or above the 30th percentile (2 cm/m^2 or more) and differed in the relative size of the right ventricle. Women with an average of 9.7 pregnancies also had left ventricle outflow tract diameters normalized for BSA (LVOTDiamIndex) in the higher ranges above the 65th percentile (1.5 cm/m^2) and healthy diastolic blood pressures at the 72nd percentile or below (89 mmHg, normal or prehypertension category (14)). Women with an average of 9.2 pregnancies had a LVOTDiamIndex below the 65th percentile, a right ventricle stroke distance (RVOTvti, the distance blood travels toward the lungs per heartbeat) above a low around the eighth percentile (12 cm), a very long marriage, and residence at the highest altitudes. Thus, one of the two very successful combinations of traits comprised relatively large left heart chambers and healthy diastolic blood pressure. The other group included women with smaller left ventricles, high blood flow values to the lungs, and the highest residence altitudes.

Regarding genomics, the LADIndex was associated with five SNPs at a significance level of 10^{-7} below the usual critical value for GWAS of $5x10^{-8}$. The minor allele effect size was +0.22 cm/m^2

\ Supporting Figure 6 below. Tree-based model of the number of pregnancies among the 364 women with complete response information.

\ Supporting Table 5 below. GWAS results for the LADIndex, a trait associated at higher values with the number of pregnancies.

Full Sample

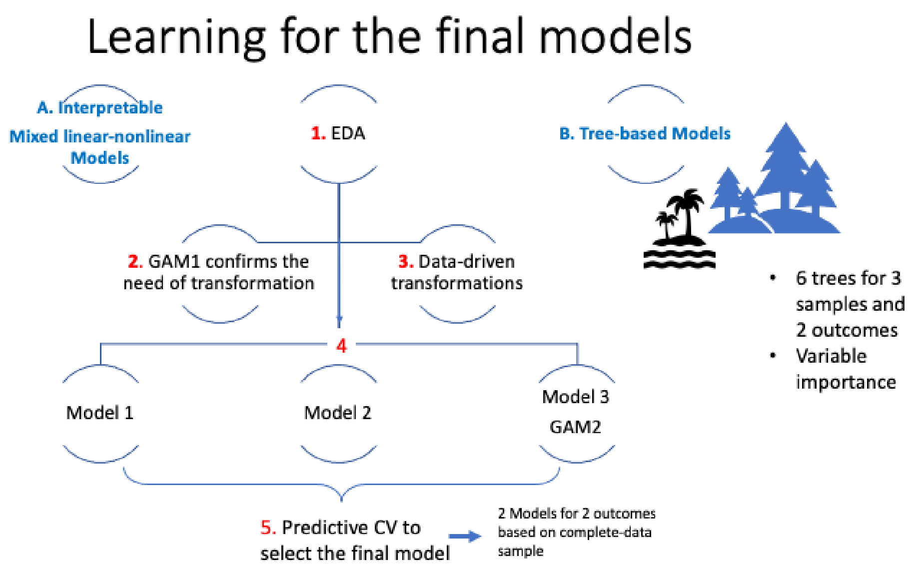
Among the *full sample* of 417 women, the tree-based model showed that those with 10 pregnancies had two or more miscarriages (Supporting Figure 7 below). The seeming contradiction of more miscarriages predicting more pregnancies arises because women return to susceptibility to conception sooner after a miscarriage than after a full-term pregnancy (likely followed by a period of lactational amenorrhea).

Small Subset without Echo or HVR Tests

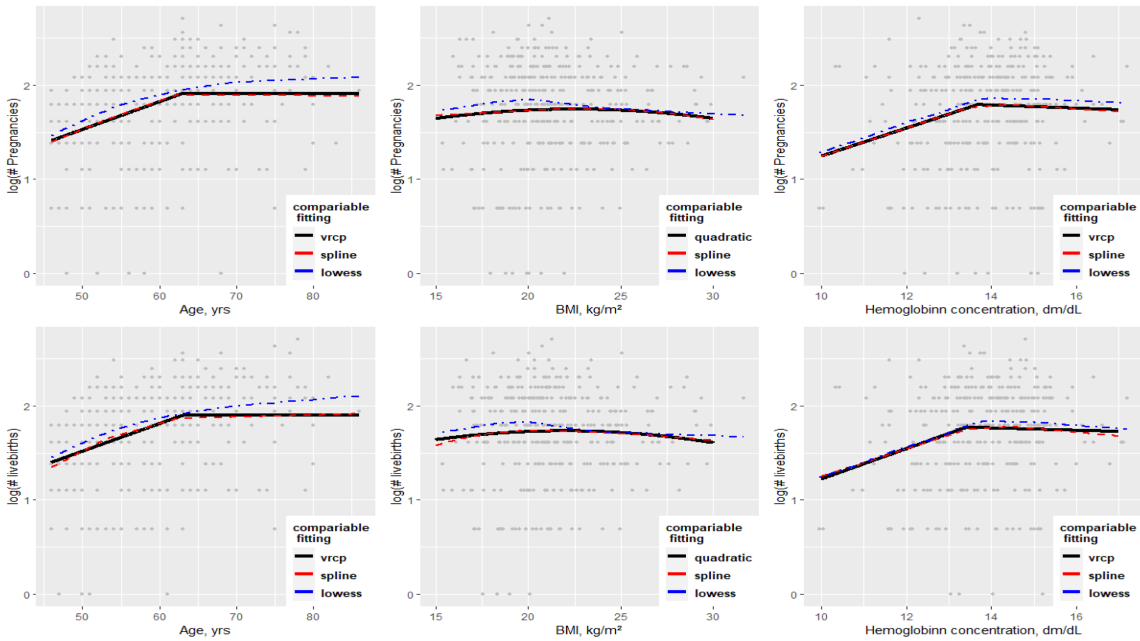
Tree-based analyses can accommodate missing data, thereby enabling examination of those who did not provide echo examinations or HVR tests. Women whose echo exams excluded them from the HVR test comprise most of this subset of 53 women (Supporting Table 1 below). Diastolic blood pressure topped the pregnancy trees as it did for livebirths (Supporting Figures 4 and 8 below). Women with the fewest pregnancies (1.6) had normal diastolic blood pressure, whereas those with the most pregnancies (8.5) had diastolic hypertension (95 mmHg or more, above the 89th percentile). As with livebirths, these trees contrast starkly with those for pregnancies in the complete sample (Supporting Figure 6 below), showing diastolic blood pressure in the hypertensive range among women with the most pregnancies.

Unsurprisingly, the significant and influential factors related to the number of pregnancies largely overlap with those for the number of livebirths. The number of pregnancies is also related to a large left atrium receiving oxygenated blood from the lungs and emptying into a large left ventricle. Three SNP sites on chromosome 12 with a minor allele effect size of + 0.023 cm/m^2 had a significance of E-07 (Table 3A).

Supporting Figures and Tables

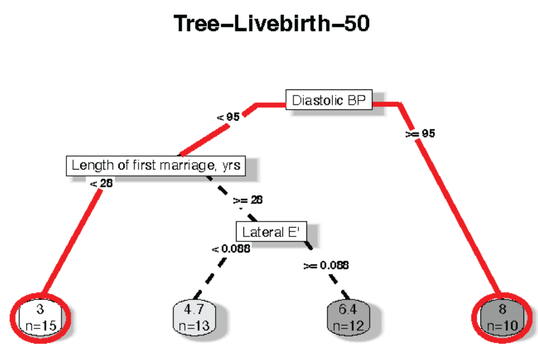


Supporting Fig. 1. Summary of data analysis steps.

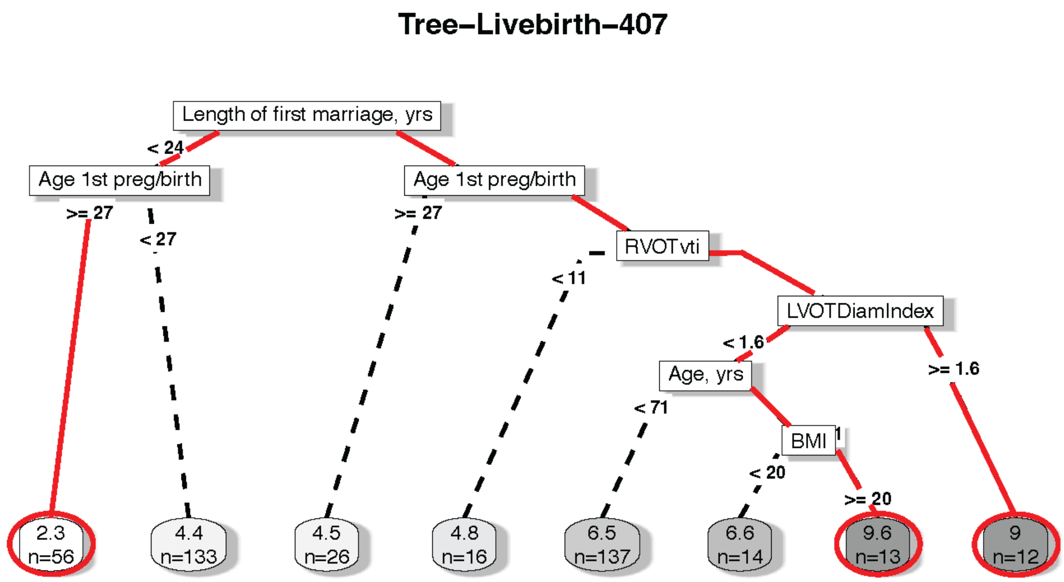


Supporting Fig. 2. Exploratory plots of the relationship of the number of pregnancies and livebirths with age, BMI, and hemoglobin.

Supporting Fig. 3. Correlations among variables describing the sample and contributing to reproductive success (columns labeled pregnum (# of pregnancies) and liveb (# of livebirths).

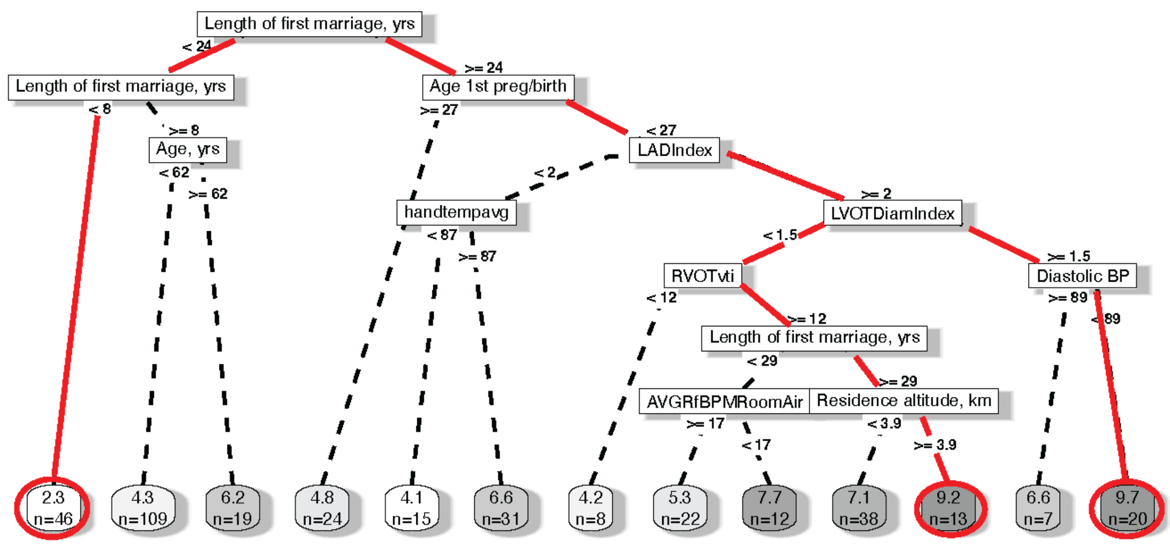


Supporting Fig. 4. Tree-based model of the number of livebirths in the subsample of 53 women without echocardiograms or hypoxic ventilatory response data. Of these, 50 had at least one livebirth.

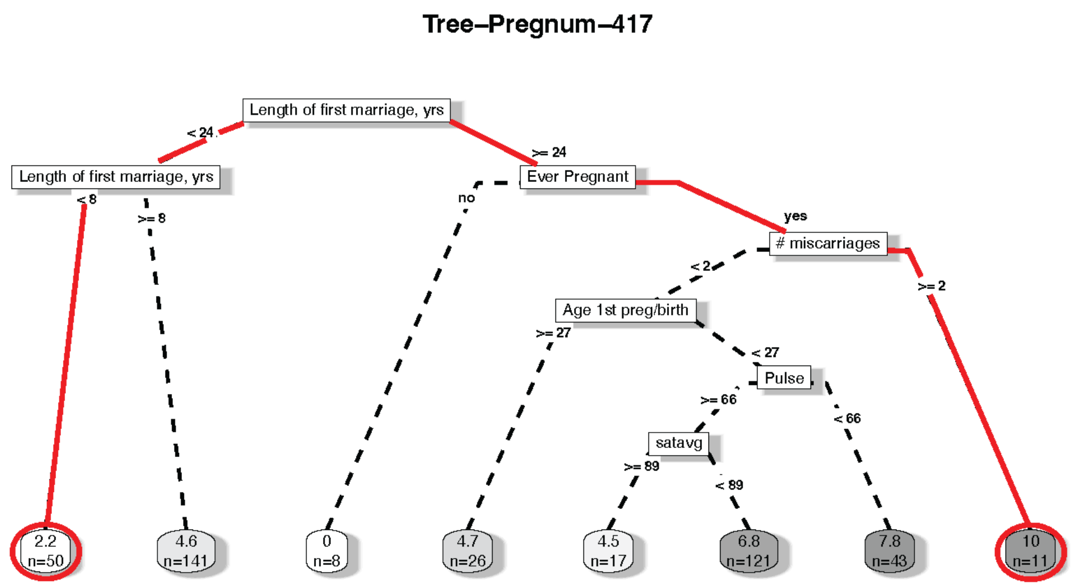


Supporting Fig. 5. Tree-based model of the number of livebirths in the full sample of 407 (those who had a livebirth).

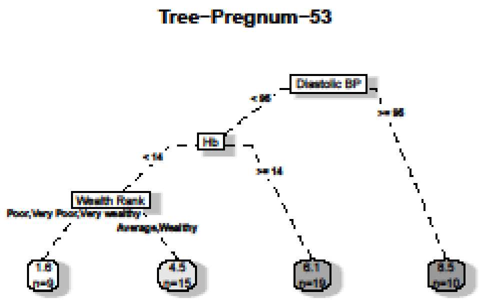
Tree–Pregnum–364



Supporting Fig. 6. Tree-based model of the number of pregnancies in the complete data sample of 364 women.



Supporting Fig. 7. Tree-based model of the number of pregnancies in the full sample of 417 women.



Supporting Fig. 8. Tree-based model of the number of pregnancies in the small subset of 53 women without echocardiograms or hypoxic ventilatory response data.

Supporting Table 1. Potential and included participants and reasons for nonparticipation or exclusion.

	Potential pool, # of women	Not in the final sample for this report, # of women
Participants from Upper Mustang in 2012	605	
# known to have died by 2019		56
# known to have moved away by 2019		45
Potential remaining participants	504	
Contacted successfully	430	
Participated partly by providing informed consent and interview data		13
Provided informed consent, interview, and physiological data	417	
Did not provide an echocardiogram or hypoxic ventilatory response data		6
Did not provide echocardiogram, did provide hypoxic ventilatory response data		12
Provided echocardiogram, did not provide hypoxic ventilatory response data		35
Provided a complete set of data	364	

Supporting Table 2. Description of variables included in the parametric and nonparametric models for the relationship between physiological and reproductive phenotypes. When calculated variables correlated highly with variables in the expression, we use the calculated value (e.g., BMI rather than height, weight, and BMI). Dividing echocardiograph values by body surface area (BSA) yields indexed variables that depend on body size.

Supporting Table 2A. Continuous variables included in the parametric and nonparametric analyses of the relationship between physiological and reproductive phenotypes.

Dependent Variables	Mean (SD)	Minimum	Median	Maximum	N	# Missing
Independent Variables						
Age, Western years	59.7 (9.2)	46	59	86	407	0 (0.0%)
Altitude of residence, km	3.8 (0.1)	3.5	3.8	4.1	407	0 (0.0%)
Direct Determinants of Exposure to Intercourse						
Length of the first marriage, years	25.1 (11.0)	2	25	53	371	36 (8.8%)
Direct Determinants of Susceptibility to Conception						
Age at first pregnancy/birth	23.8 (4.2)	14	23	43	407	0 (0.0%)
Age at last pregnancy/birth	36.1 (6.0)	21	37	50	405	2 (0.5%)
Physiological Variables						
Hemoglobin concentration, gm/dL	13.8 (1.3)	8.4	13.9	17.7	404	3 (0.7%)
% Oxygen saturation of hemoglobin (fingertip, transmittance device)	86.2 (4.1)	65	86.7	94.3	404	3 (0.7%)
Pulse, f/m	72.7 (9.1)	47.7	72	105.3	404	3 (0.7%)
Body mass index, kg/m^2	21.2 (3.1)	13.7	20.8	31.6	407	0 (0.0%)
Systolic blood pressure, mmHg	126.3 (22.4)	82	122.7	218	407	0 (0.0%)
Diastolic blood pressure, mmHg	83 (12.2)	51.3	81.3	132.7	407	0 (0.0%)
Aural temperature, ^{o}F	97.8 (1.0)	93.8	97.9	100.1	401	6 (1.5%)
Hand Temperature, ^{o}F	87.8 (3.1)	72	88.3	95	404	3 (0.7%)

Finger perfusion index	7.5 (3.1)	1.2	7.3	17.6	404	3 (0.7%)
Forced expiratory volume, 6 sec, L/min	2.7 (0.7)	0.9	2.7	5.5	390	17 (4.2%)
Echocardiography Variables						
Heart rate, bpm	66.9 (8.3)	39	65.5	114	390	17(4.2%)
Left ventricle fractional shortening, %	32.3 (3.9)	21	32	46	389	18(4.4%)
Right ventricular outflow tract peak velocity, m/s	0.7 (0.2)	0.3	0.7	1.4	387	20 (4.9%)
Right ventricular outflow tract velocity time interval, cm	16.3 (3.8)	7.9	15.9	30.7	386	21 (5.2%)
RVTOTPACC, right ventricular outflow tract pulmonary artery acceleration time, cm/sec	79.2 (30.1)	7	79	187.3	385	22 (5.4%)
LVEA, LVEwave early diastolic transmitral velocity, m/s + LVAwave LV late diastolic trans mitral velocity, m/s	1.1 (0.3)	0.5	1	2	384	23 (5.7%)
Left ventricular outflow tract velocity time integral, cm	20.4 (4.2)	9.8	20.1	52.4	381	26 (6.4%)
Right ventricular outflow tract short axis, cm	2.4 (0.3)	1.5	2.4	3.6	385	22 (5.4%)
LATERALE, lateral E' early diastolic transmitral flow tissue velocity, m/sec	0.1 (0)	0	0.1	0.2	368	39 (9.6%)
MEDIALE, medial e' early diastolic mitral annular tissue velocity, m/sec	0.1 (0)	0	0.1	0.2	370	37 (9.1%)
MitralEe Ratio, ratio of tissue to blood flow across the mitral valve	8.8 (2.5)	3.9	8.3	24.8	366	41 (10.1%)
LVOTDiamIndex, cm/m^2	1.4 (0.2)	1	1.4	2.1	386	21 (5.2%)
AoDIndex, aortic root diameter, cm/m^2	2.5 (0.3)	1.8	2.5	3.8	387	20 (4.9%)
LVEDDIndex, left ventricular end diastolic diameter, cm/m^2	3 (0.3)	1.9	3	3.9	388	19 (4.7%)
LVESDIndex, left ventricular end systolic diameter, cm/m^2	2 (0.3)	0.5	2	2.7	387	20 (4.9%)
LADIndex, left atrial diameter, cm/m^2	2.2 (0.4)	1.5	2.2	3.6	387	20 (4.9%)
RV1Index, right ventricle width at the base, cm/m^2	2.5 (0.4)	1.5	2.5	4.9	387	20 (4.9%)
RV2Index, right ventricle width at the midpoint, cm/m^2	1.8 (0.3)	1	1.8	3.1	387	20 (4.9%)
CILeft, cardiac index, left heart, cm/m^2	3.1 (0.8)	1.3	3	9.1	377	30 (7.4%)
CIRight, cardiac index, right heart, cm/m^2	3.5 (1.2)	1.2	3.3	9.7	381	26 (6.4%)
Ventilation and Hypoxic Ventilatory and Heart Rate Response						

HVR, hypoxic ventilatory response, ΔL/ΔSat(forehead)	0.2 (0.2)	-0.1	0.2	1.5	366	41 (10.1%)
HHRR, hypoxic heart rate response, ΔHR/min/ΔSat by reflectance (below)	0.7 (0.3)	0.1	0.7	2.4	364	43 (10.6%)
Respiratory frequency in room air, f/min	19.1 (3.3)	9.6	18.8	29.4	368	39 (9.6%)
% Saturation hemoglobin, forehead (reflectance)	91 (3.8)	74.9	91.6	99.6	368	39 (9.6%)
End-tidal CO_2, mmHg	31.3 (2.5)	21.6	31.6	38.3	368	39 (9.6%)
AVGVtBTPSLRoomAir, tidal volume in room air, BTPS, L	0.6 (0.1)	0.4	0.6	1.1	366	41 (10.1%)

Supporting Table 2B. Categorical variables (count, percent, and missing).

Independent Variables		**Count**	**Percent**	**# Missing**
Indirect Determinants of Reproductive Success				
Type of marriage	Never married	24	5.9%	0 (0.0%)
	Not married to a cousin, not polyandrous	324	79.6%	
	Not married to cousin and polyandrous	31	7.6%	
	Married to a cousin, not polyandrous	24	5.9%	
	Married to cousin and polyandrous	4	1.0%	
Household relative wealth rank in 2022, quintiles	Very poor	37	9.7%	25 (6.1%)
	Poor	74	19.4%	
	Average	97	25.4%	
	Wealthy	127	33.2%	
	Very wealthy	47	12.3%	
Woman and partner both had some education	Yes	141	34.6%	0 (0.0%)
	No	266	65.4%	
Direct Determinants of Exposure to Intercourse				
Current marital status	Currently married	233	57.2%	0 (0.0%)
	Divorced/Separated	17	4.2%	
	Never married	15	3.7%	
	Remarried	4	1.0%	

	Widowed	138	33.9%	
Married continuously from 25 to 40 years of age	Yes	225	55.5%	0 (0.0%)
	No	182	44.2%	
Pregnancy History				
# of stillbirths	0	364	89.4%	0 (0.0%)
	1	40	9.8%	
	2	2	0.5%	
	3	1	0.2%	
# of miscarriages	0	349	85.7%	0 (0.0%)
	1	43	10.6%	
	2	14	3.4%	
	3	1	0.2%	
# of twin pregnancies	0	387	95.1%	0 (0.0%)
	1	19	4.7%	
	2	1	0.2%	
Direct Determinants of Susceptibility to Conception				
Ever used contraception	Yes	179	44.1%	1 (0.2%)
	No	227	55.9%	

Supporting Table 2C. Repeatability of influential predictor variables measured three ways as intraclass correlations (two-way, mixed effect model with absolute agreement), within-subject coefficients of variation, and the limits of agreement, n=21. Reference (5) reports the repeatability of hemoglobin concentration, oxygen saturation (fingertip), and pulse.

Variables with a repeat measurement on a later day	Intraclass correlation, 95% CI of two measurements	Within-subject coefficient of variation, mean ± SD	Average height differences and limits of agreement (2 SD (15))
Height, m	0.99 [0.98, 1.00]	0.00± 0.005	0.0, 0.28
Weight, kg	0.94 [-0.05, 0.99]	0.06 ± 0.024	3.6, 2.8
Diastolic blood pressure, mmHg	0.86 [0.67, 0.94]	0.06 ± 0.042	2.5, 14.32
BSA, m^2	0.96 [0.02, 0.99]	0.02 ± 0.012	0.05, 0.04
LVOTDiameter, cm	0.77 [0.45, 0.91]	0.02 ± 0.012	2.0, 0.44
LVOTDiameter Index	0.82 [0.34, 0.94]	0.06 ± 0.046	-0.1, 2.48
LAD, cm	0.85 [0.64, 0.94]	0.06 ± 0.046	-0.5, 0.64
LAD Index	0.80 [0.49, 0.92]	0.06 ± 0.055	-0.1, 0.46
RVOTvti, m/sec	0.67 [0.19, 0.87]	0.14 ± 0.111	-1.3, 8.12
Aural temperature, °F	0.72 [0.49, 0.89]	0.00 ± 0.003	0.1, 1.48
Lateral E', m/sec	0.79 [0.49, 0.92]	0.12 ± 0.097	0.0, 0.043
HHRR, ∆HR/min/∆Sat by reflectance	0.83 [0.59, 0.93]	0.16 ± 0.123	-0.0, 0.47
Oxygen saturation (forehead reflectance), %	0.92 [0.81, 0.97]	0.14 ± 0.111	-0.5, 4.4
Respiratory frequency, f/min (n=8)	0.84 [0.31, 0.97]	0.07 ± 0.045	1.2, 4.44

Supporting Table 3. Comparisons of the present sample collected in 2019 and the larger sample collected in 2012 (reported in (1-5, 16-19)).

Supporting Table 3A. Characteristics measured in 2012 of survivors, deaths, and emigrants between 2012 and 2012.

	Survivors to 2019	Died between 2012 and 2019	Emigrated between 2012 and 2019
Values in 2012	mean ± SD	mean ± SD	mean ± SD
Age, y	52.9 ± 9.43	63.4 ± 10.48	56.3 ± 10.12
Hemoglobin concentration, gm/dL	13.8 + 1.43	14.0 + 1.18	14.0 + 1.43
% of oxygen saturation (fingertip)	86.4 ± 4.34	84.2 ± 4.3	85.5 ± 5.71
Pulse, bpm	74.3 ± 10.91	75.3 ± 12.35	76.9 ± 12.82
# pregnancies	5.3 ± 2.82	5.3 ± 2.77	5.3 ± 3.18
# livebirths	5.1 ± 2.72	5.5 ± 2.86	5.0 ± 2.75

Supporting Table 3B. The 2012 and 2019 samples closely resemble one another. The women are older and more are widowed in 2019.

	2012	2019
N	605	417
Age	54.2 + 10.2, 39 - 88	58.8 ± 9.26, 46-86
% widowed	24%	34%
Had used contraception	38 %	43%
# pregnancies	5.4 + 2.83, 0-15	5.3 ± 2.82, 0-15
# livebirths	5.1 + 2.75, 0 - 14	5.2 ± 2.82, 0-14
Age at first pregnancy	23.9 + 4.38, 16-43	23.8 ± 4.22, 14-43
Length of first marriage	24.3 + 13.2, 2-62	25 ± 11.3, 2-53, n=396
Hemoglobin concentration, gm/dL	13.8 + 1.4, 6.6 – 18.3	13.8 ± 1.30, 8.4-17.7
Percentage of oxygen saturation of hemoglobin (fingertip plethysmography)	86.2 + 4.5, 69-95	86.2 ± 4.09, 65.0-94.0
Pulse	74.4 + 11.2, 51 - 121	72 ± 9.3, 48 – 105 (n=421)

Supporting Table 4. Model performance characteristics for cross-validation analyses. Model 1' performs the best.

	Model 1'	Model 2'	Model 3' – semiparametric model
m1=Mean of 100 mMRSE	**3.394208**	3.515711	3.844044
m2=Var of 100 mMRSE	**0.001702063**	0.001994447	0.009398326
m3=Mean of 100 vMRSE	**0.8112960**	0.8556203	1.0446332

Supporting Table 5. General linear model analysis of the number of pregnancies for 364 women (who had echocardiography and hypoxic ventilatory response information) based on covariates in Table 1. Quasi R^2 = 0.50, AIC=1304.7.

$$\begin{aligned} log(\#preg) \sim\ & -3.65 - 0.05 * b1mage + 0.01 * lmrge + 0.92 * age1 + 0.12 * LVESD \\ & -0.35 * HHRR + 0.03 * SatRmAir - 0.39 * mc + 0.00075 * RVOTvti \\ & +1.83 * cntvY + 0.90 * Hb1 + 0.056 * BMI1 \\ & +0.01 * LVESD * BMI1 + 0.42 * HHRR * cntvY - 0.00087 * SatRmAir * BMI1 \\ & +0.033 * mc * RVOTvti - 1.17 * cntvY * Hb1 \end{aligned}$$

age1 = $f_{age}(age)$, representing a linear function of the age until age 63 and then staying flat; BMI1= $f_{BMI}(BMI)$, a quadratic function; Hb1= $f_{hb}(hb)$, two segments with high values in the middle, all in Equation (1) above, and the P-values for the significant variables are as follows:

Variable	b1mage	lmrge	age1	HHRR	SatRmAir	cntvY	Hb1	HHRR* CntvY	mc*RVOTvti	cntvY *Hb1
P-value	**<10^{-10}**	**0.0002**	$\mathbf{5 * 10^{-5}}$	**0.0086**	**0.001**	**0.040**	**0.0088**	**0.009**	**0.017**	**0.021**

Here, HHRR* CntvY represents the interaction term between HHRR and those who used contraceptives.

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