BioCycle

Eunice Kennedy Shriver NICHD National Institute of Child Health & Human Development

Racial differences in menstrual cycle patterns of sex hormones

An application of harmonic models

Edwina Yeung (yeungedw@mail.nih.gov)
Epidemiology Branch
Division of Epidemiology, Statistics, and Prevention Research
Eunice Kennedy Shriver National Institute of Child Health and Human Development

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BioCycle

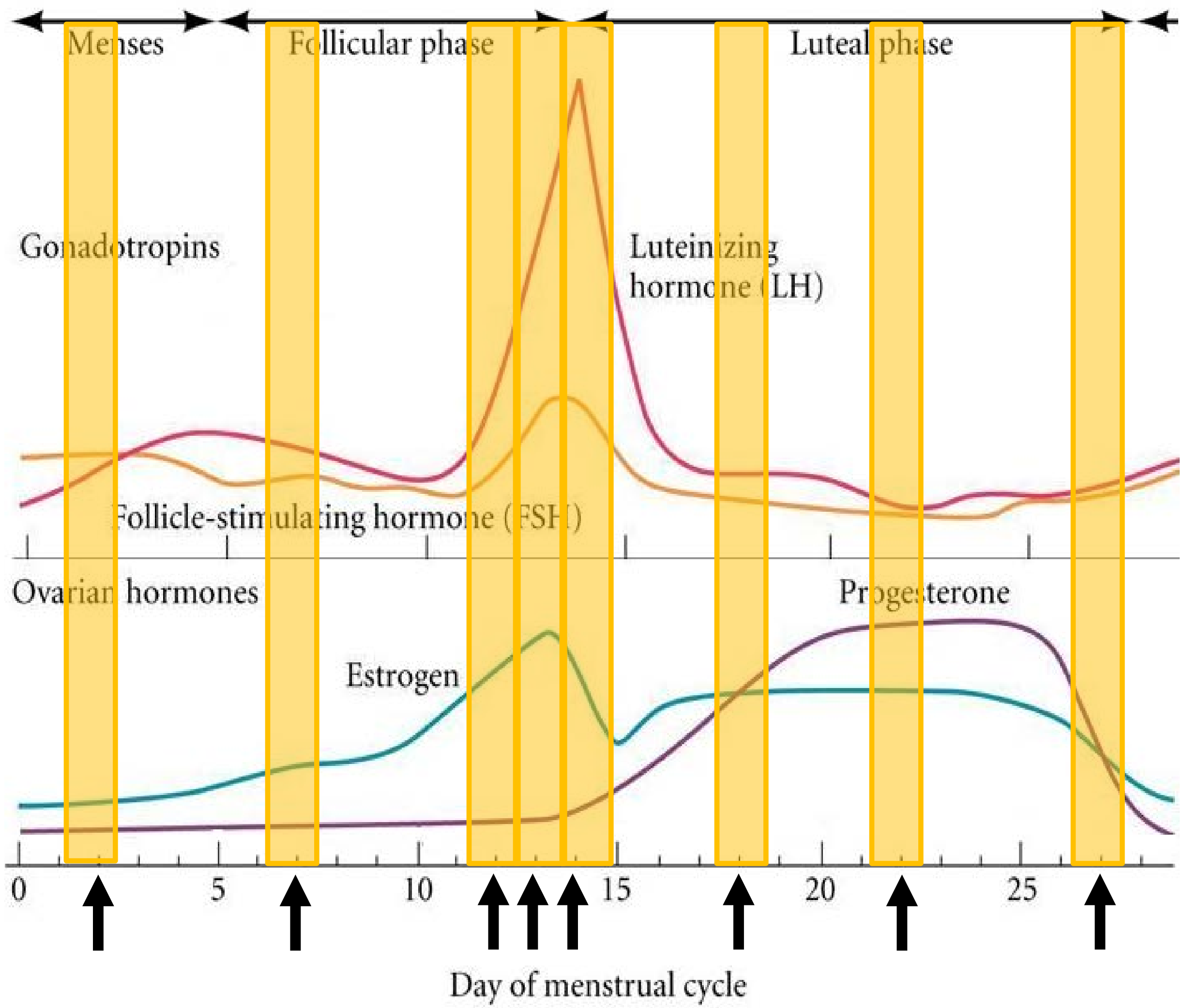
Study Design

- Prospective cohort study to assess relation between biomarkers of oxidative stress & reproductive hormones across two menstrual cycles

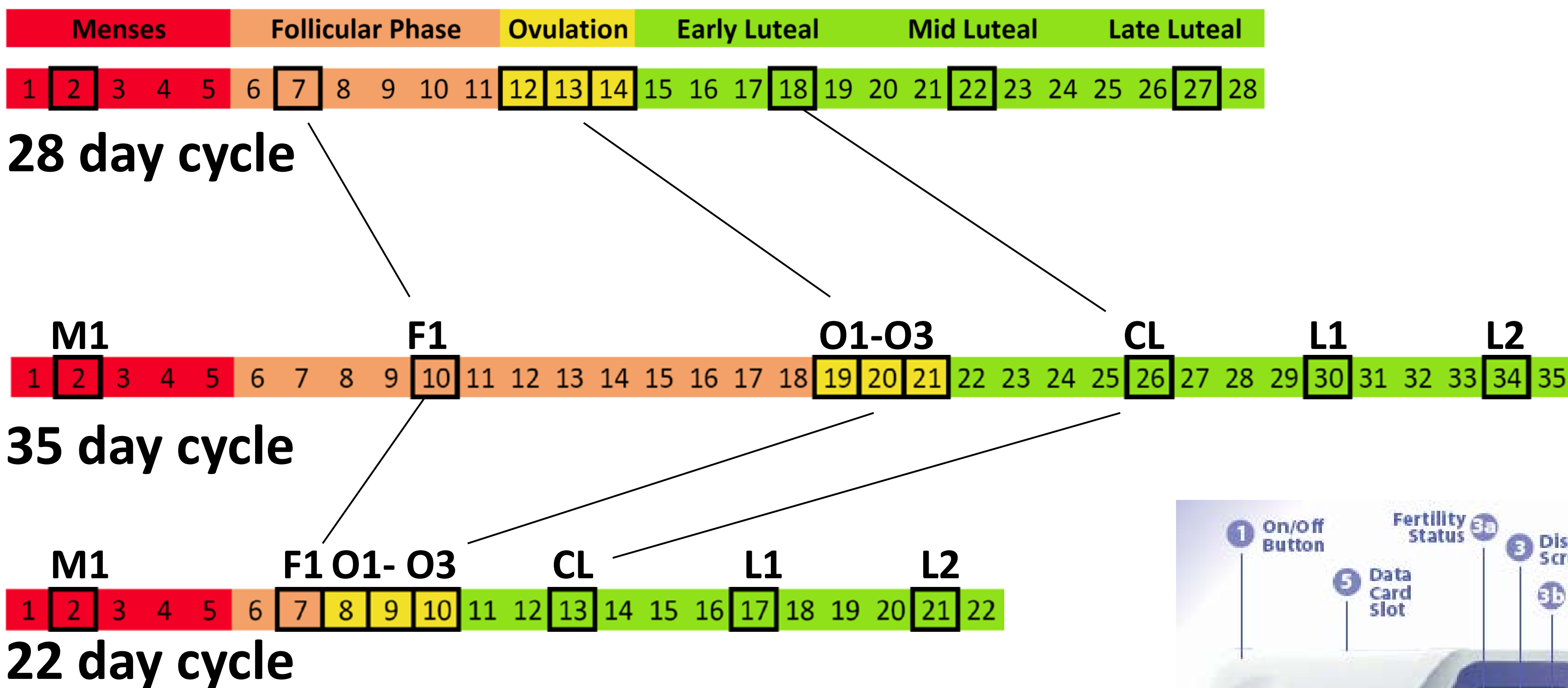
- Healthy, normally menstruating premenopausal women
  - Aged 18-44 years
  - Self-reported cycle length between 21 and 35 days for each menstrual cycle for the past 6 months

- Exclusion Criteria
  - OC use in past 3 months
  - Self reported BMI <18 or >35
  - On a restricted diet
  - On lipid lowering medications
  - Hx menstrual & ovulation disorders
  - Hx gastrointestinal conditions
  - Hx chronic diseases

BioCycle Study Design (n=259)



Menstrual Cycle Variation
Biological time: M1, F1, O1, O2, O3, CL, L1, L2



Decisions prior to analysis…

1. Time scale

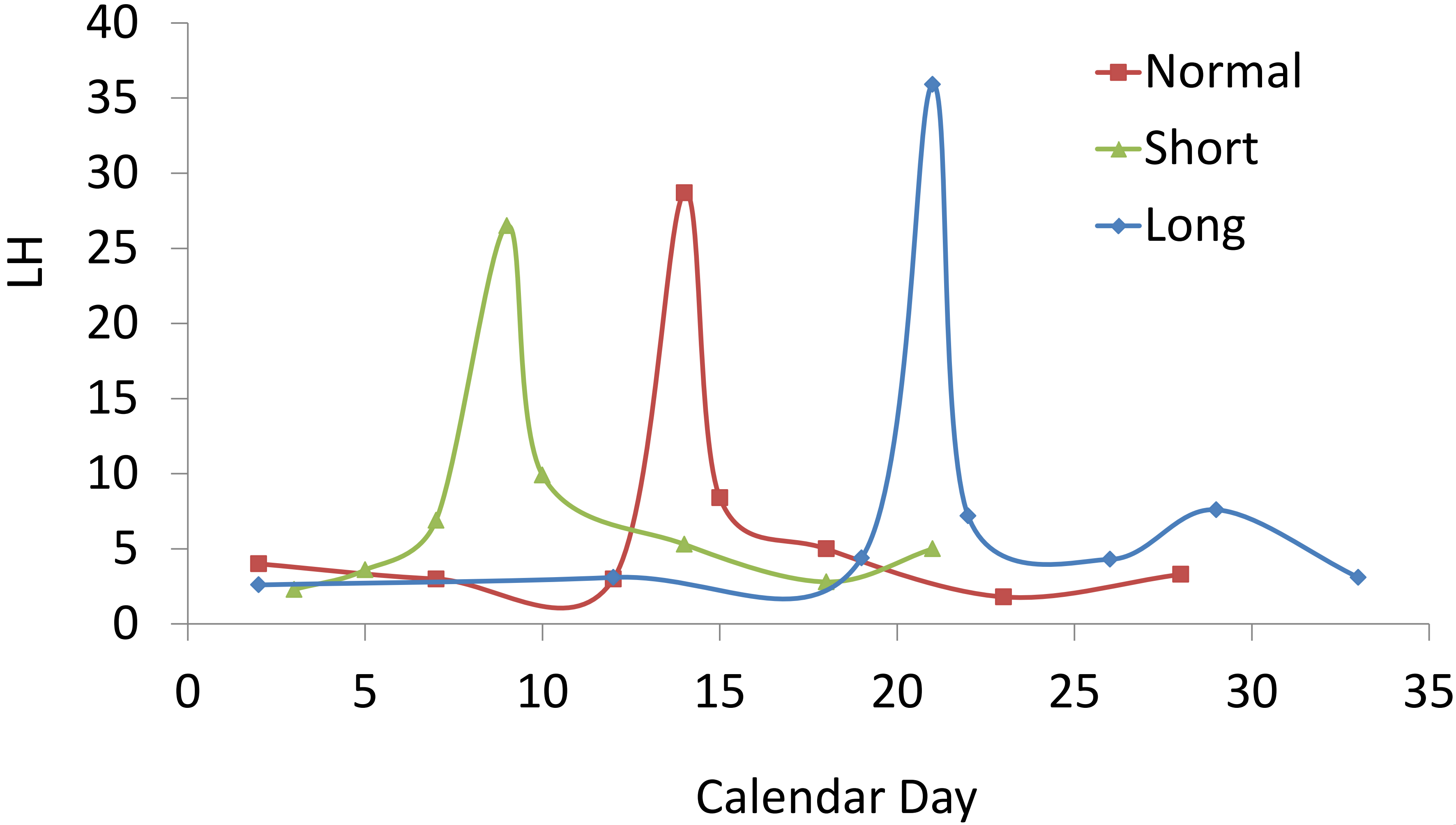
2. Number of Harmonics

Modeling Time

- Actual visit days (calendar time)
- Scheduled visit days (biological time)
- Actual visit days standardized by cycle length
- Registered cycles (centered on ovulation)

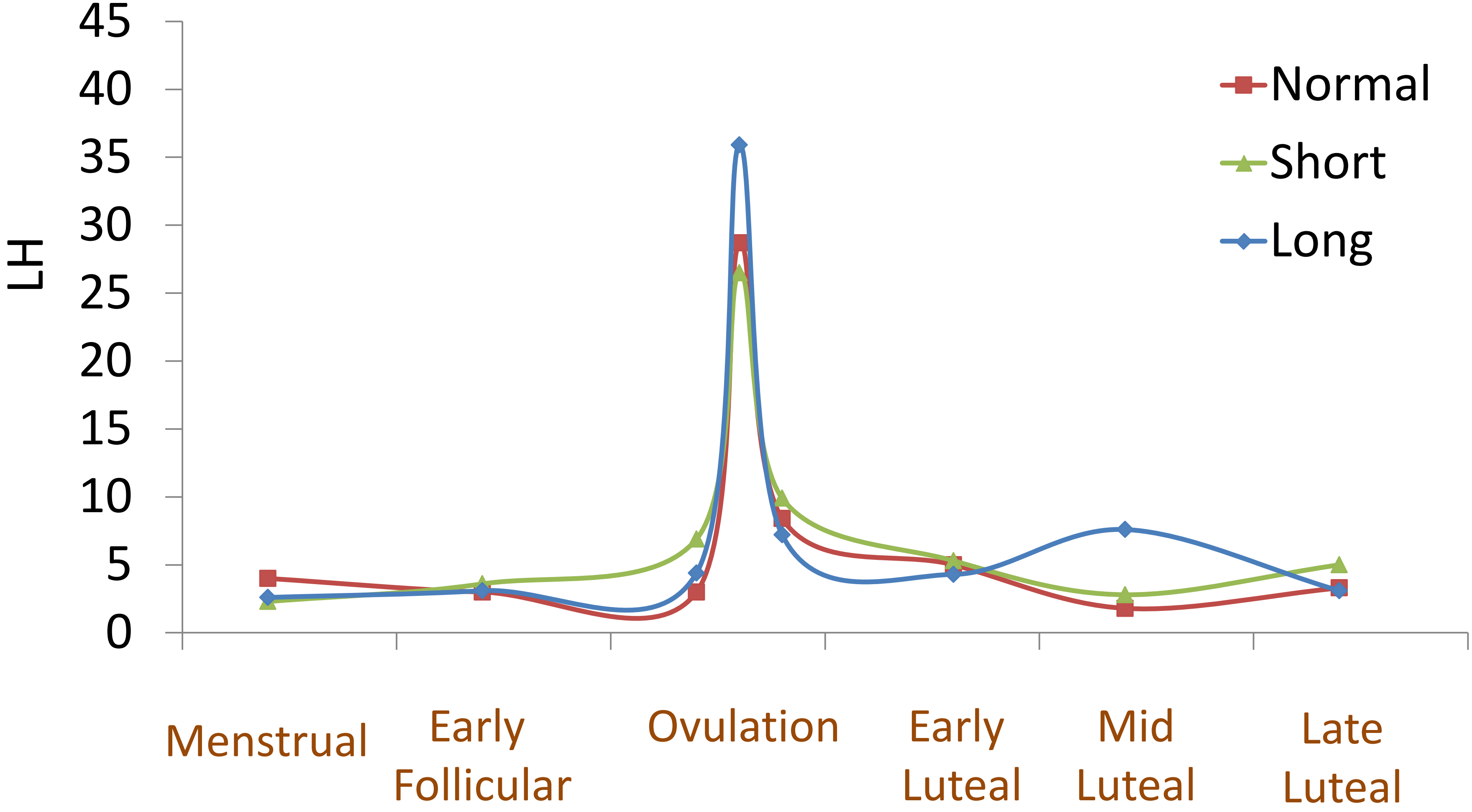
Actual visit days (Calendar Time)

an example of 3 cycles of varying lengths



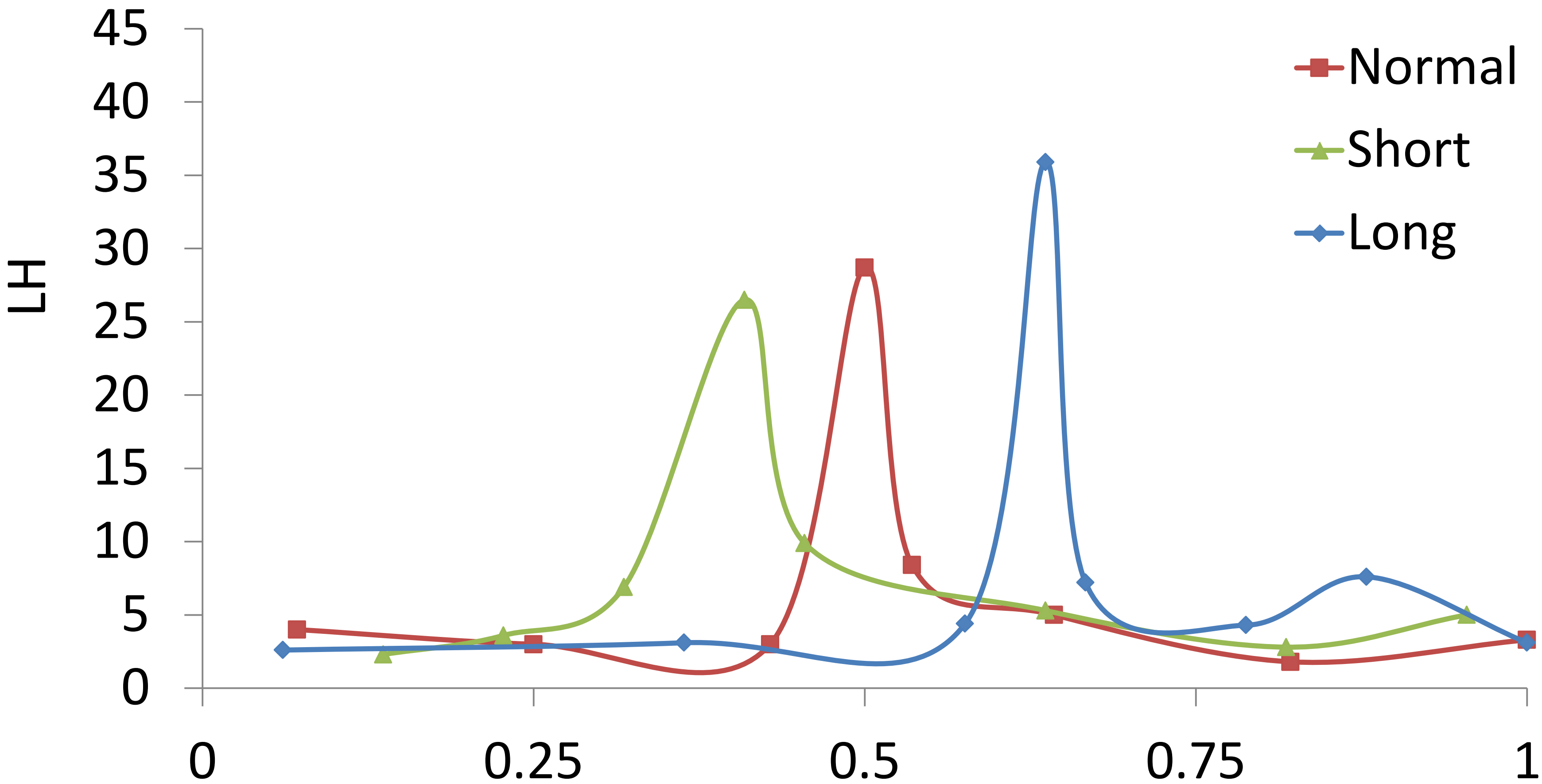
Scheduled visit days (biological time)
M1, F1, O1, O2, O3, CL, L1, L2

an example of 3 cycles of varying lengths



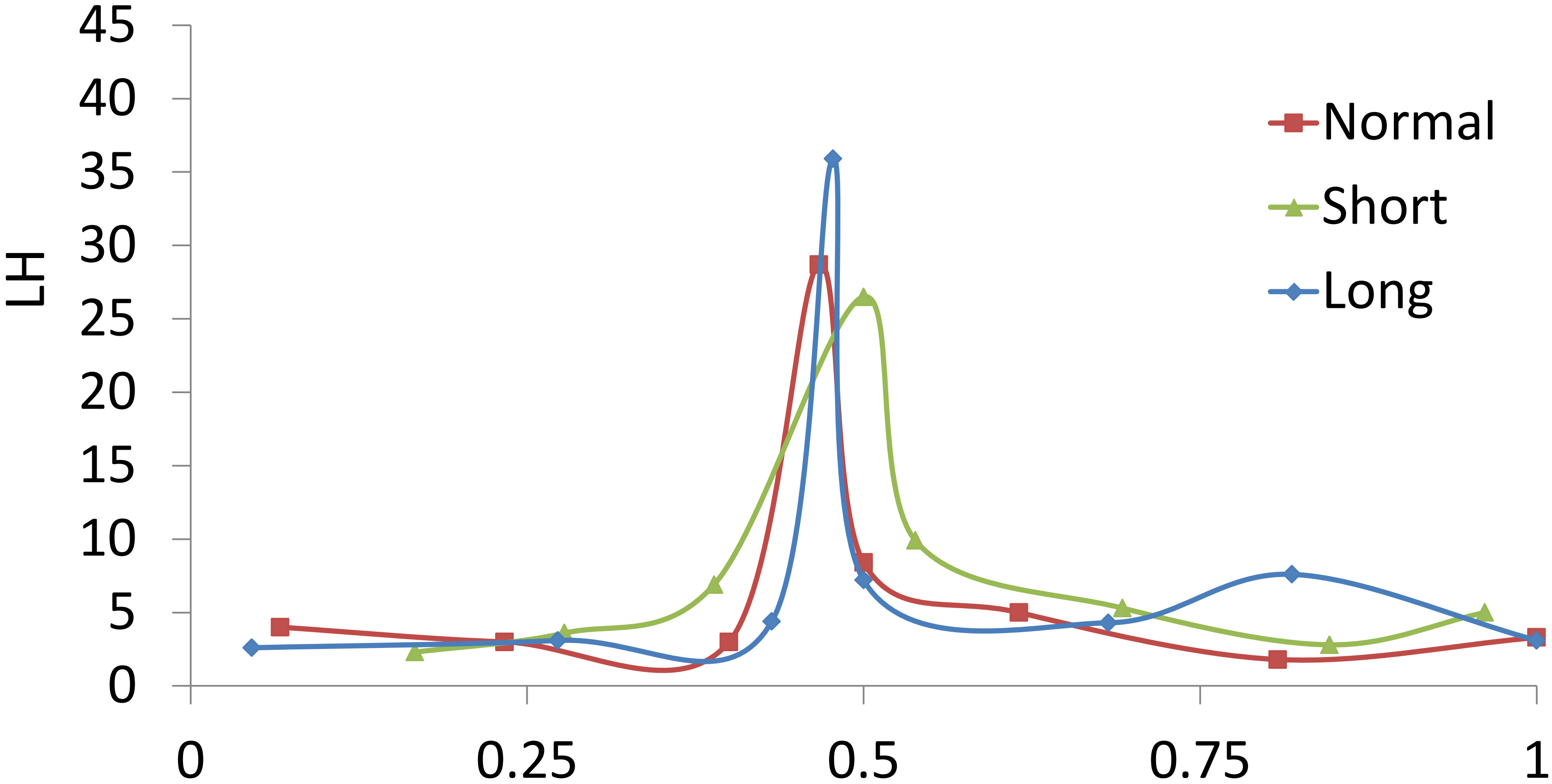
Actual visit days standardized by cycle length

an example of 3 cycles of varying lengths



Registered cycles (centered on ovulation)

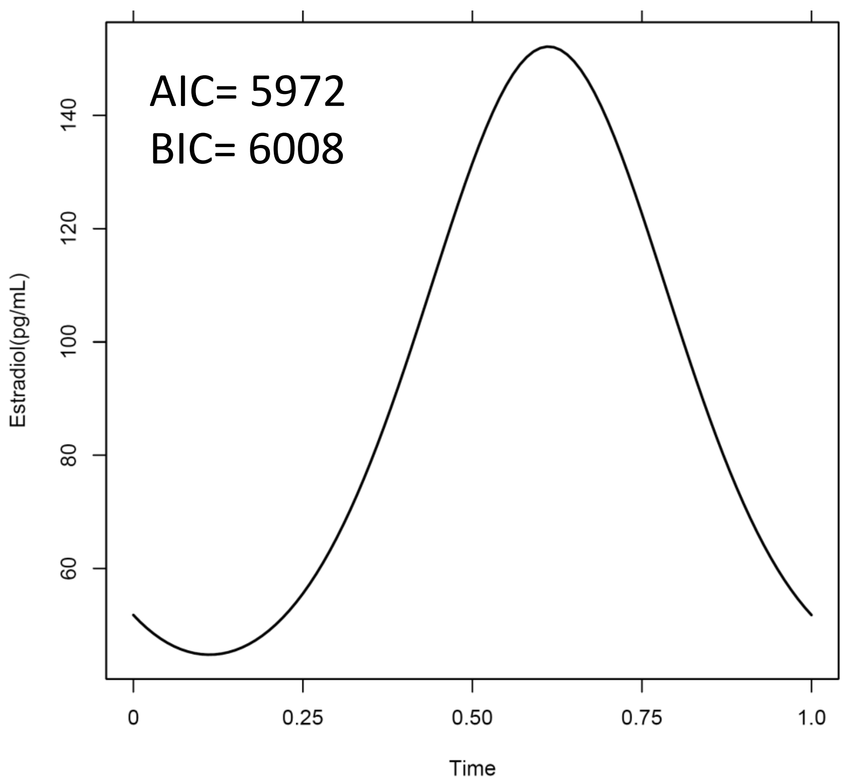
an example of 3 cycles of varying lengths



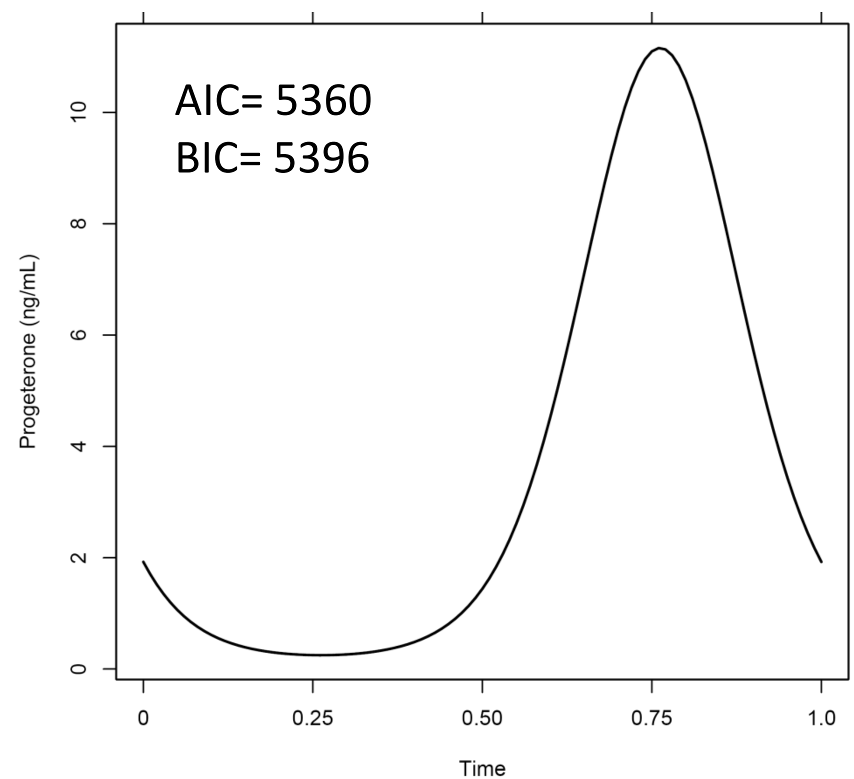
Choosing the number of harmonic terms

One Harmonic Term

Estradiol

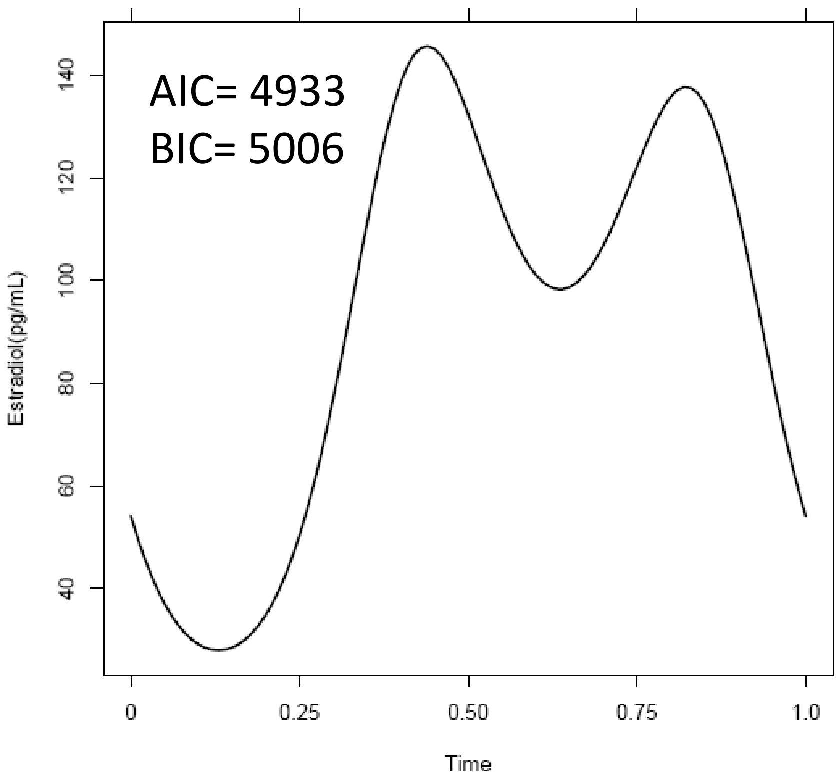


Progesterone

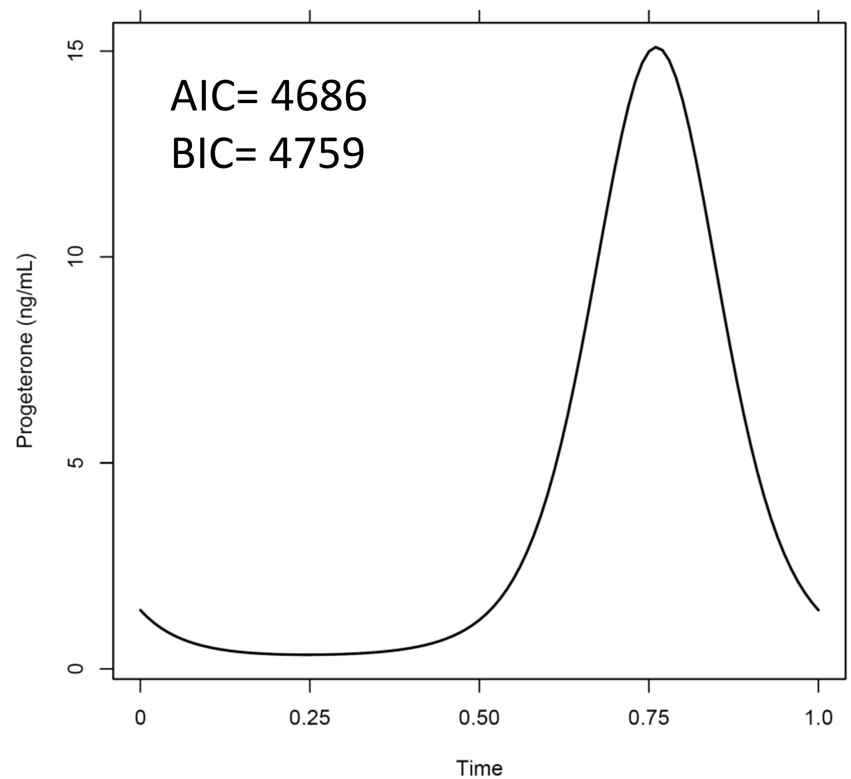


Two Harmonic Terms

Estradiol

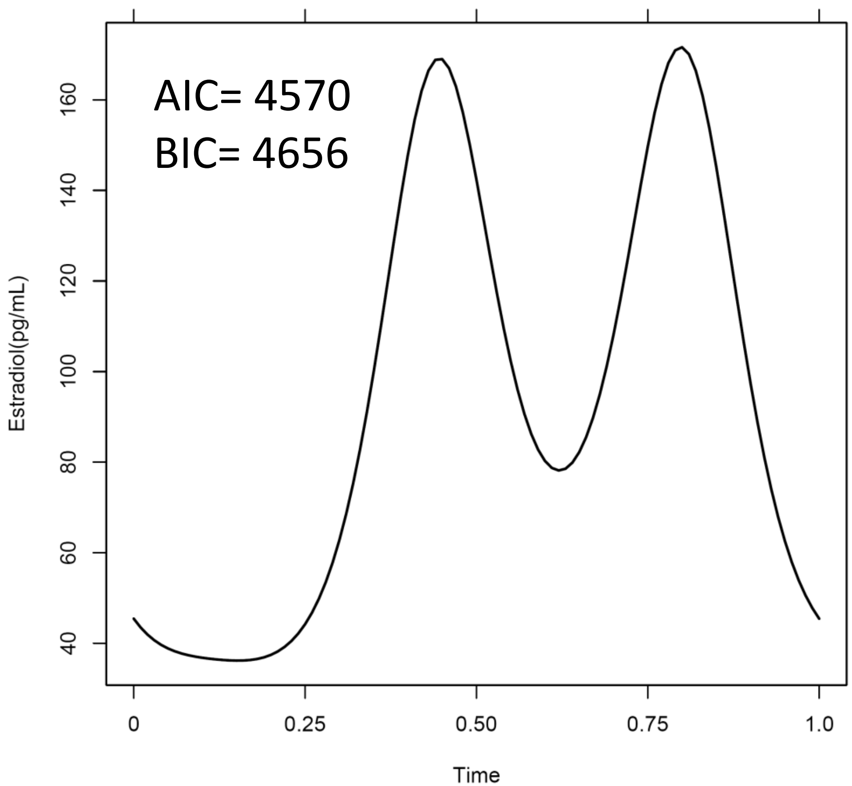


Progesterone

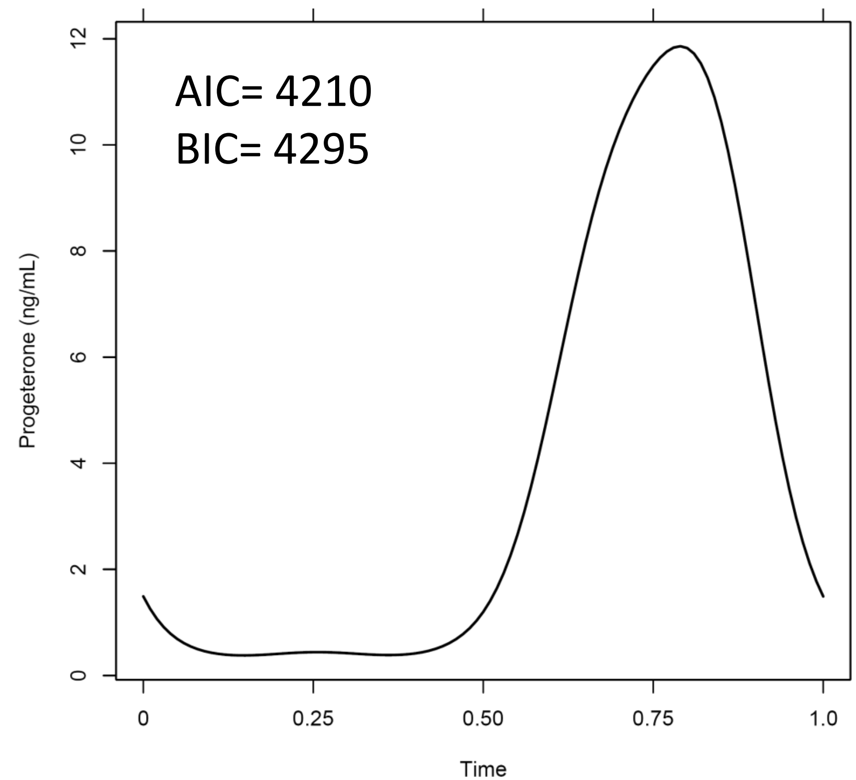


Three Harmonic Terms

Estradiol

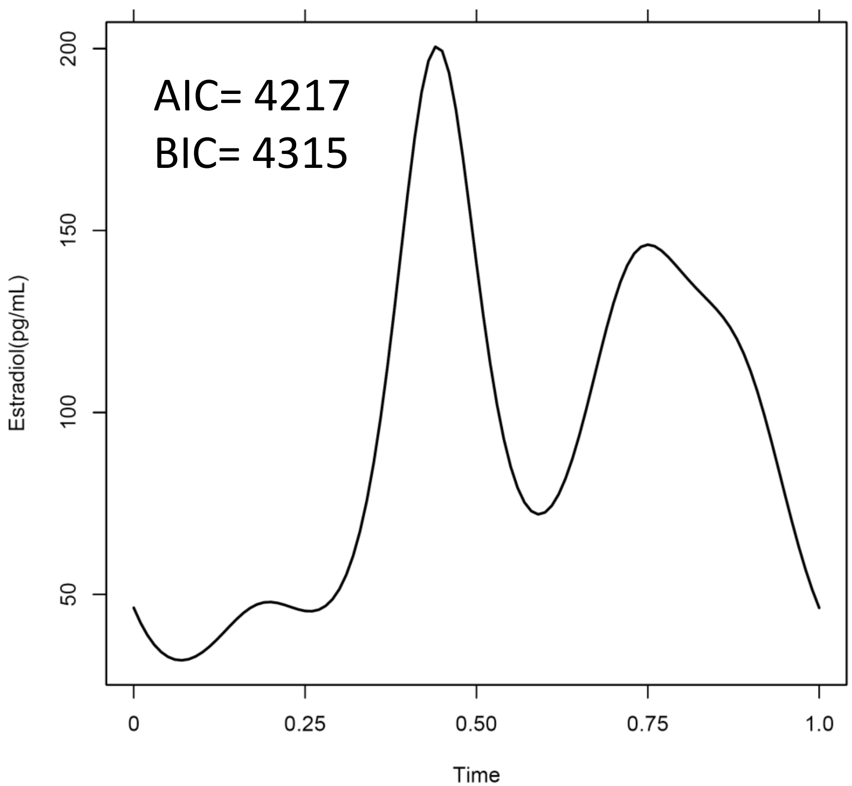


Progesterone

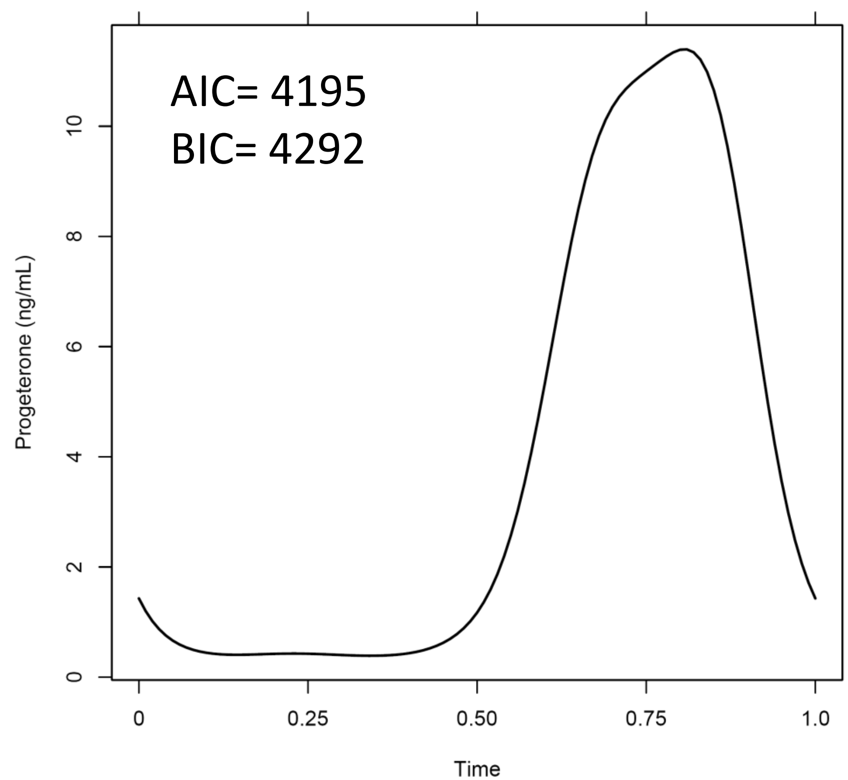


Four Harmonic Terms

Estradiol

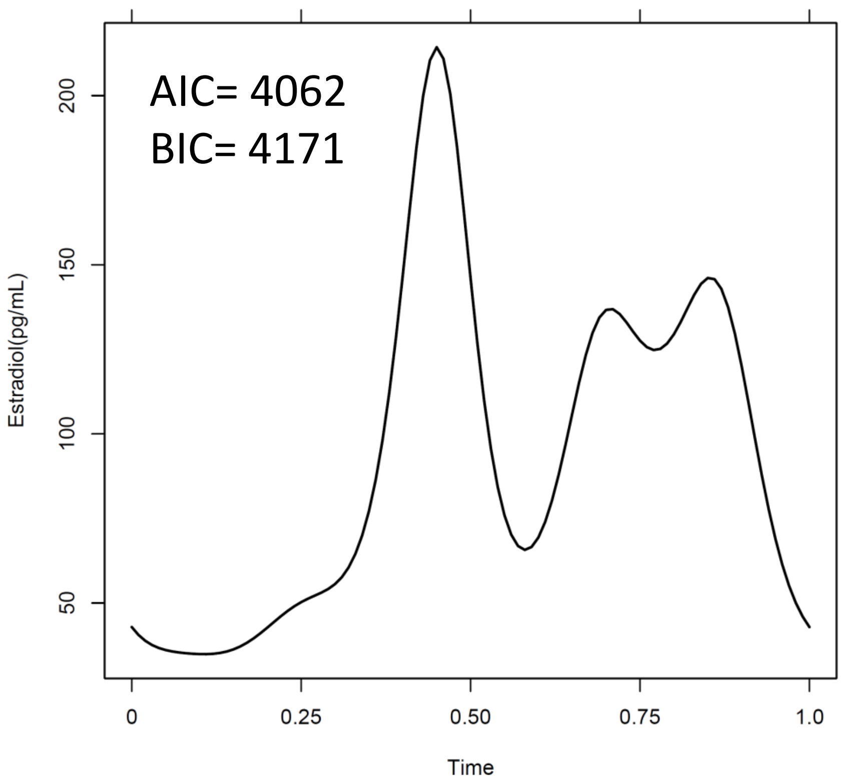


Progesterone

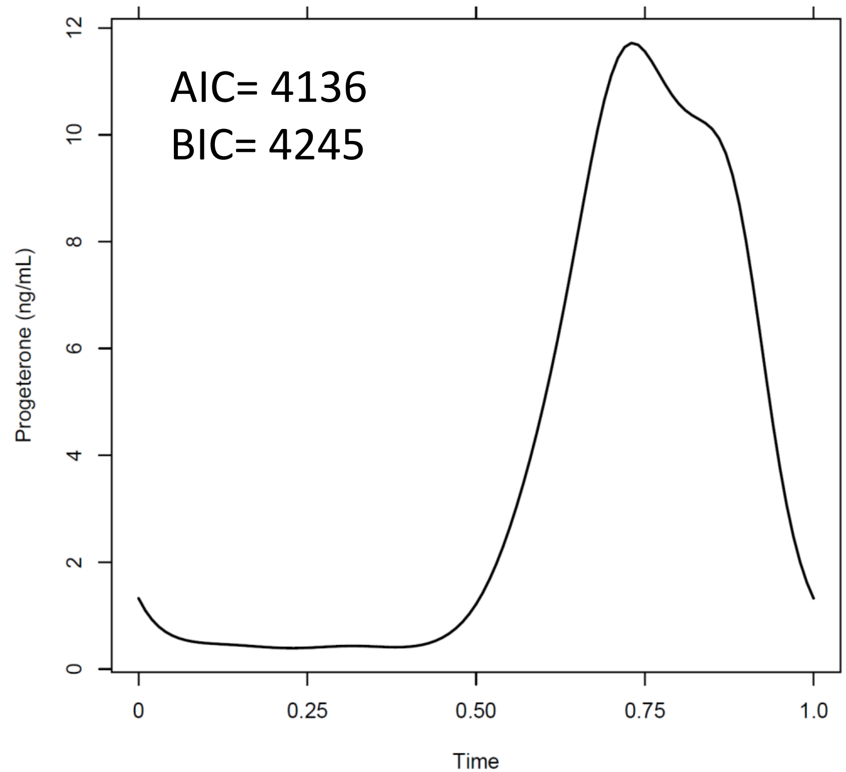


Five Harmonic Terms

Estradiol



Progesterone



Summary on Modeling

1. Time
   – Registered cycles - standardized to cycle length and centered on day of ovulation
2. Harmonics
   – Optimal to use 4 harmonic terms to model the sex hormones and SHBG

Analysis of racial differences in menstrual cycle patterns

Background

- Racial differences in sex hormone patterns
- Importance:
  - Breast cancer
  - Cardiovascular disease
  - Fertility
- Little research done on amplitude and phase shift differences

Rebbek et al. Menopause 2010 (PMID: 20505544)
Setiawan et al. Cancer Epidemiology Biomarkers 2006 (PMID: 17035391)
Freeman et al. Fertility and Sterility 2005. (PMID: 17035391)

Racial Distribution in BioCycle Study

	Original cohort	Analysis cohort
Self-reported at baseline	n=259	n=248
White	154 (59%)	147 (57%)
Black	51 (20%)	50 (19%)
Asian Indian	13 (5%)	11 (4%)
East Asian (including Filipino, Chinese, Korean, Japanese)	27 (10%)	26 (10%)
Other (including Puerto Rican, Hawaiian, American Indian, Middle Eastern)	14 (6%)	14 (5%)

N	248
Age (year)	27.6 (8)
Age at menarche (year)	12.4 (1)
Household Income (%)	
Less than $19,999	21.5%
$20,000-$39,999	24.4%
$40,000-$74,999	26.8%
$75,000-$99,999	17.5%
$100,000 or over	9.8%
Education (%)	
High school or less	12.5%
Some College	37.9%
Bachelor/Associates	38.3%
Graduate Program	11.3%
Married (%)	26.0%
Weight (kg)	65 (11)
Height (cm)	164 (6)
BMI (kg/m2)	24.2 (4)

Methods

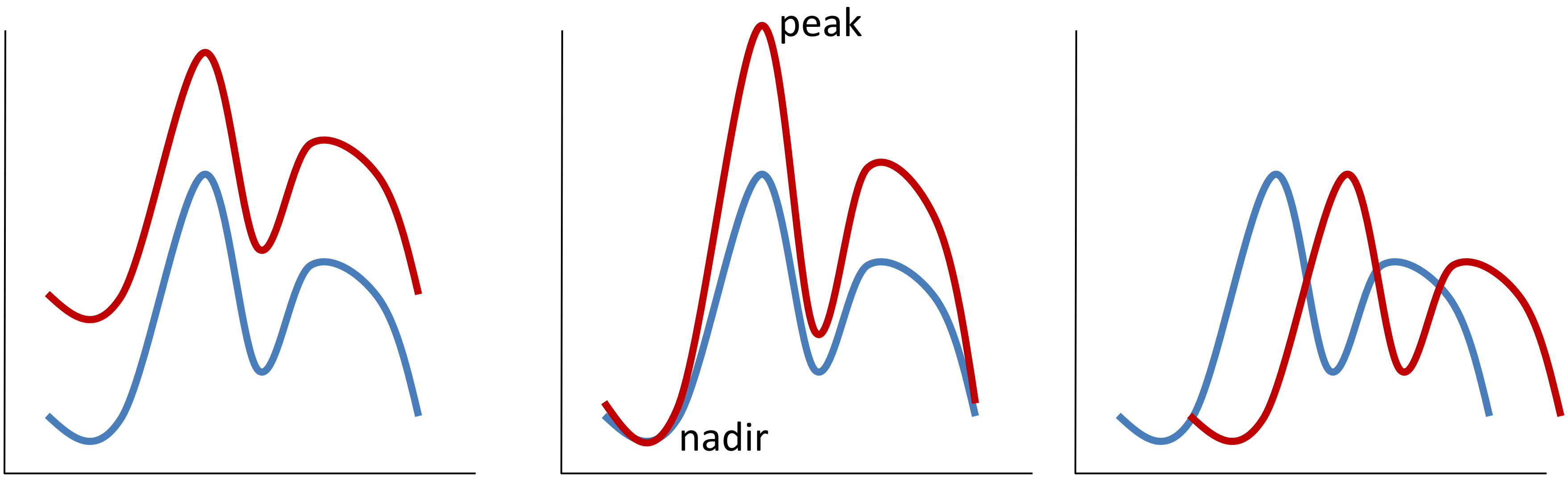
- Log-transformed hormone levels
  – Back-transformed in figures (geometric mean)
- Models
  – Unadjusted associations by categories of race (reference group = white women)
  – Adjust for age, BMI

Nonlinear Mixed Models with Harmonic Terms

Albert and Hunsberger - Biometrics 2005

$$y_{ijk} = \phi_{1ik} + \exp(\phi_{2ik})\, f\{t_{ijk} / T_{ik} - a \log it(\phi_{3ik})\} + \varepsilon_{ijk}$$

Mean **Amplitude** **Phase Shift**



Output – fixed effects for log(E2)

Variable	Category	Value	Std.Error	t-value	p-value
A.(Intercept)	White	4.309	0.131	32.8	<0.0001
A.mrace1	Black	0.208	0.049	4.2	<0.0001
A.mrace2	Asian Indian	0.215	0.090	2.4	0.02
A.mrace3	East Asian	-0.041	0.063	-0.6	0.52
A.mrace4	Other	0.095	0.080	1.2	0.23
A.screenage		0.005	0.002	2.0	0.04
A.BMI		-0.004	0.005	-0.8	0.43
B.(Intercept)	White	-0.529	0.113	-4.7	<0.0001
B.mrace1	Black	0.099	0.041	2.4	0.02
B.mrace2	Asian Indian	0.085	0.078	1.1	0.28
B.mrace3	East Asian	0.025	0.054	0.5	0.64
B.mrace4	Other	-0.092	0.074	-1.2	0.21
B.screenage		0.000	0.002	-0.2	0.87
B.BMI		0.000	0.004	-0.1	0.93
C.(Intercept)	White	0.555	0.035	15.9	0.00
C.mrace1	Black	-0.010	0.012	-0.8	0.41
C.mrace2	Asian Indian	-0.006	0.022	-0.3	0.79
C.mrace3	East Asian	0.025	0.016	1.6	0.11
C.mrace4	Other	0.021	0.022	0.9	0.34
C.screenage		-0.004	0.001	-7.1	<0.0001
C.BMI		0.003	0.001	2.8	<0.0001

Transforming coefficients

$$y_{ij} = \phi_{1i} + \exp(\phi_{2i}) f\{t_{ij} - a\log it(\phi_{3i})\} + \varepsilon_{ij}$$

- Φ_{1i} = A = mean difference

$$A1 + A0 - (A0) = A1$$

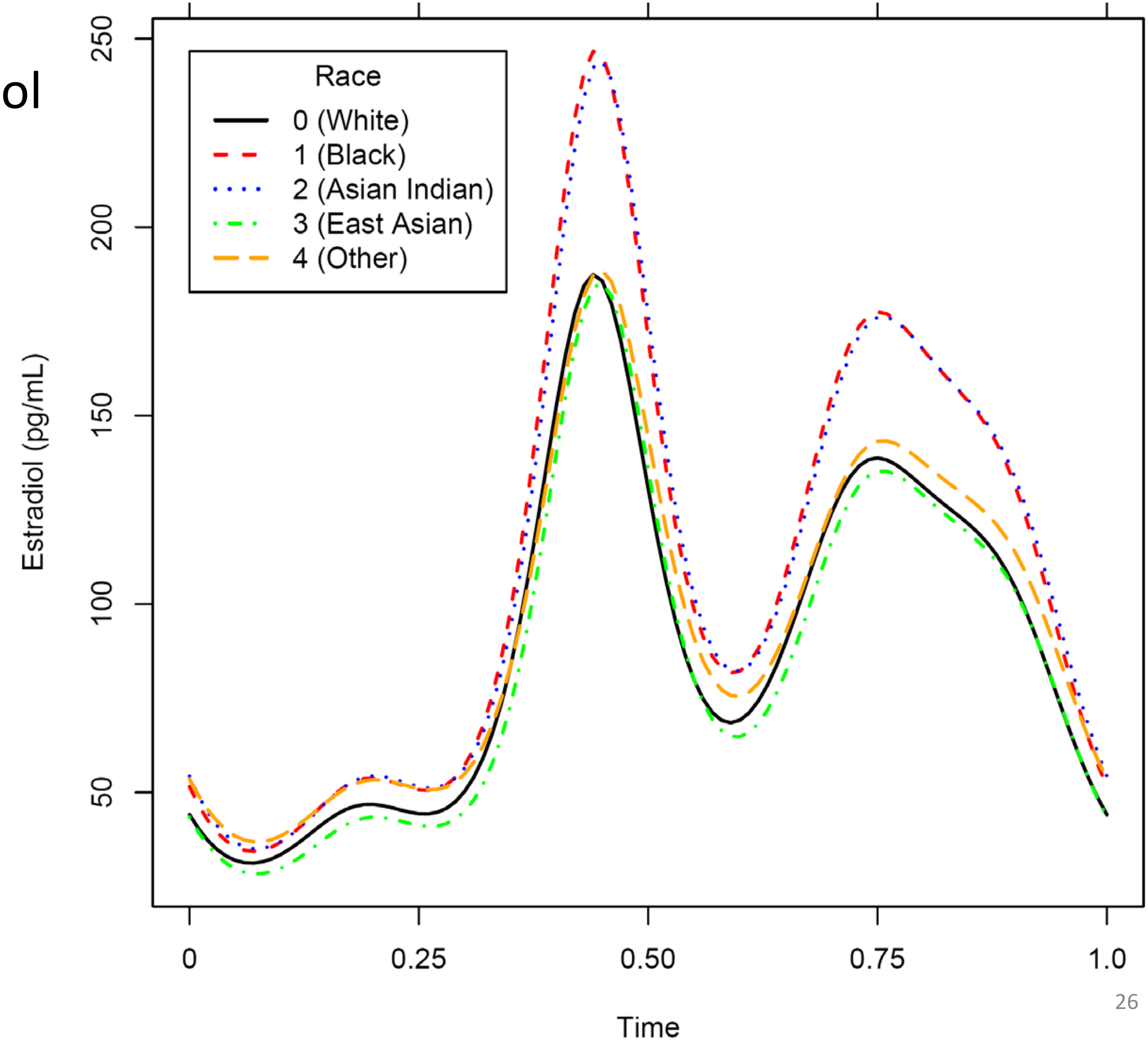
- Φ_{2i} = B: amplitude
  - Absolute difference or % difference

$$e^{B1+B0} - e^{B1} \qquad\qquad e^{B1}$$

- Φ_{3i} = C: Phase shift

$$\frac{e^{C1+C0}}{1+e^{C1+C0}} - \frac{e^{C0}}{1+e^{C0}}$$

Estradiol

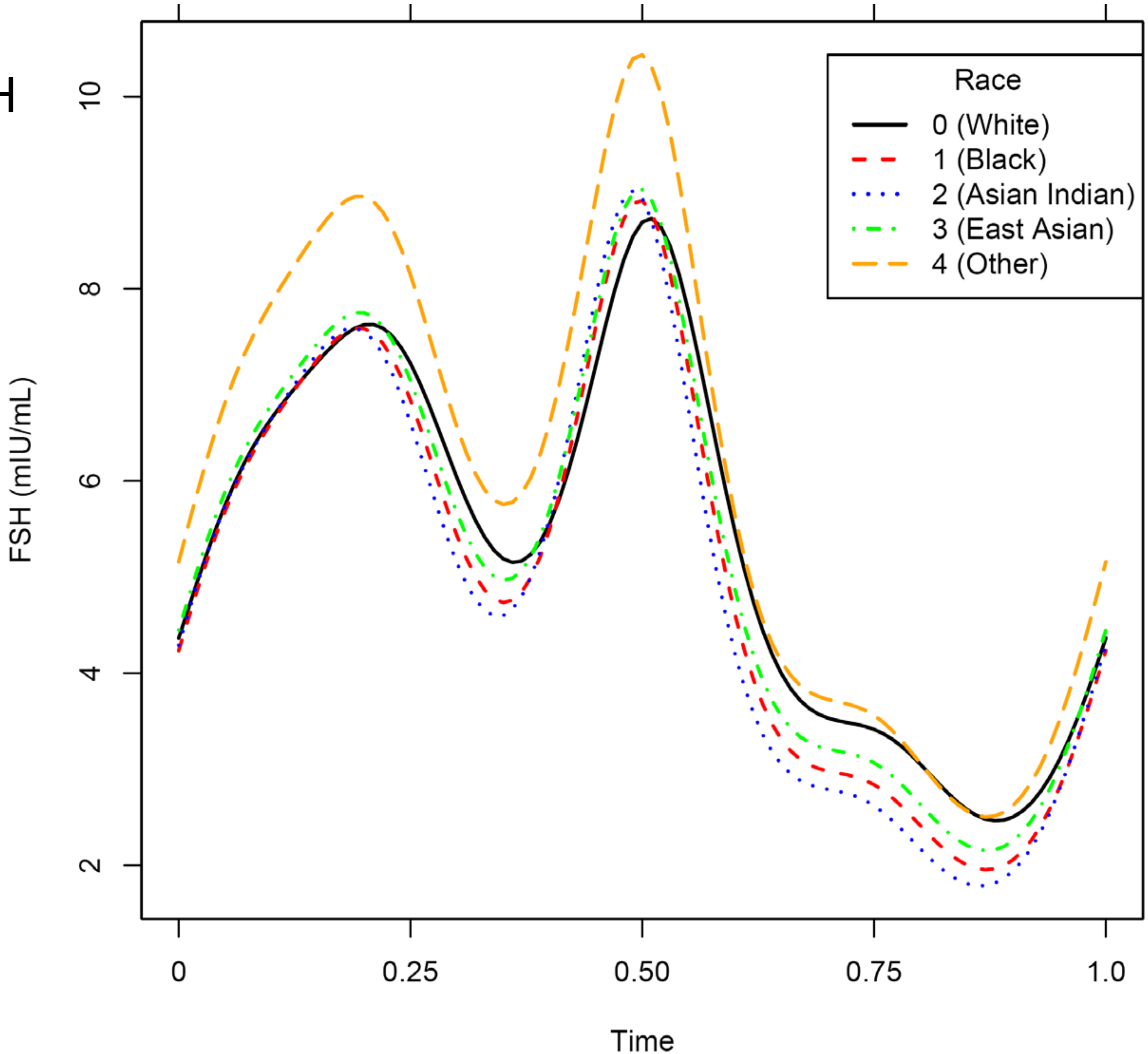


Race
0 (White)
1 (Black)
2 (Asian Indian)
3 (East Asian)
4 (Other)
Estradiol (pg/mL)
250
200
150
100
50
0
0.25
0.50
0.75
1.0
Time

Mean (SE) E2 levels by race

Race	**n**	**Unadjusted**	**p-value**	**AGE + BMI**	**p-value**
White	147	4.35 (0.02)		4.31 (0.13)	
Black	50	**0.19 (0.05)**	**0.0001**	**0.21 (0.05)**	**<0.0001**
Asian Indian	11	**0.19 (0.09)**	**0.03**	**0.22 (0.09)**	**0.02**
East Asian	26	-0.05 (0.06)	0.41	-0.04 (0.06)	0.52
Other	14	0.08 (0.08)	0.30	0.10 (0.08)	0.23

FSH

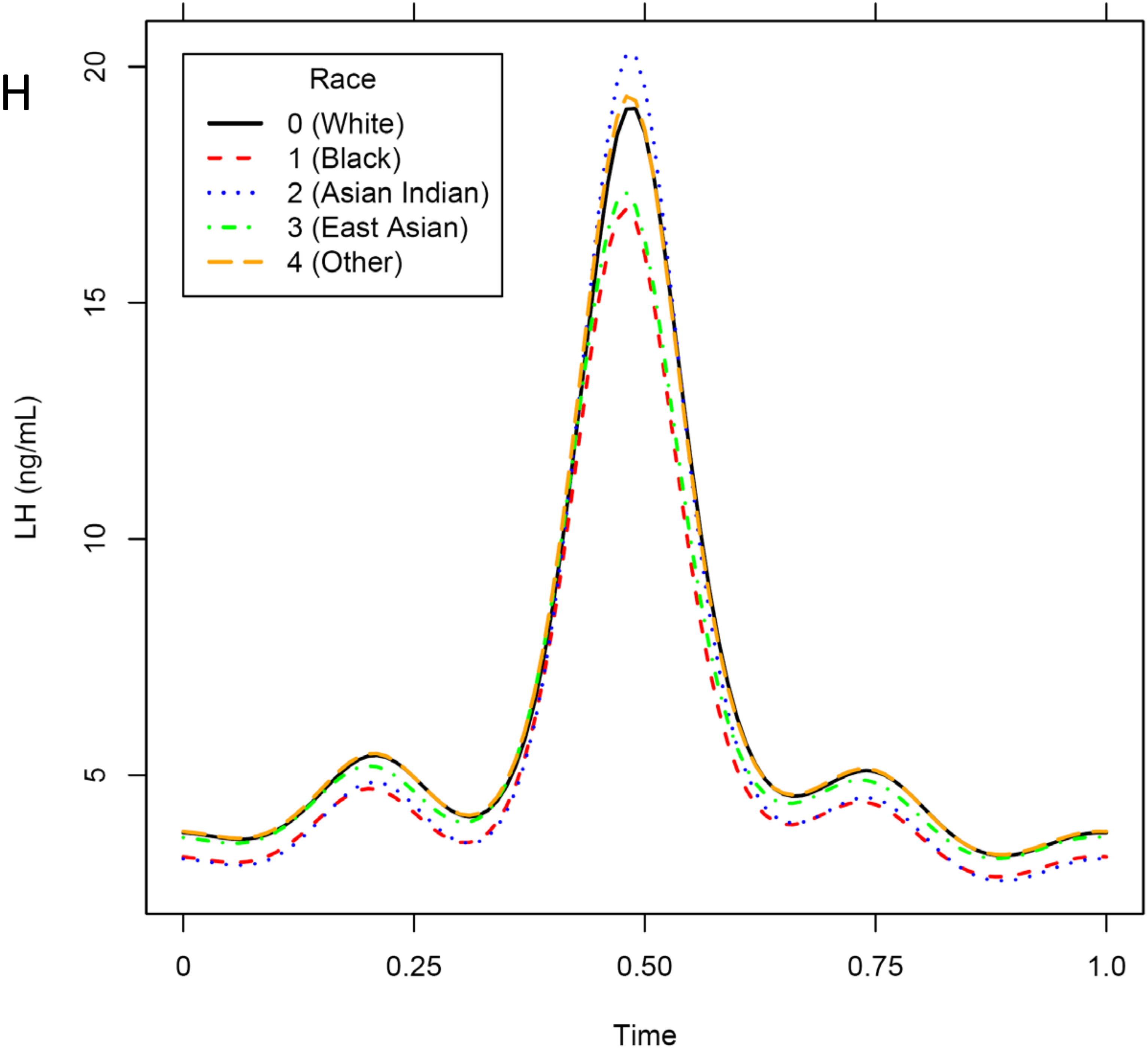


Race
0 (White)
1 (Black)
2 (Asian Indian)
3 (East Asian)
4 (Other)
FSH (mIU/mL)
2
4
6
8
10
0
0.25
0.50
0.75
1.0
Time

Mean (SE) FSH levels by race

Race	n	Unadjusted	p-value	AGE + BMI	p-value
White	147	1.60 (0.03)		1.63 (0.12)	
Black	50	-0.09 (0.05)	0.08	-0.01 (0.05)	0.75
Asian Indian	11	-0.12 (0.09)	0.19	-0.06 (0.08)	0.51
East Asian	26	-0.04 (0.06)	0.52	-0.03 (0.06)	0.62
Other	14	0.10 (0.08)	0.23	0.13 (0.07)	0.08

LH

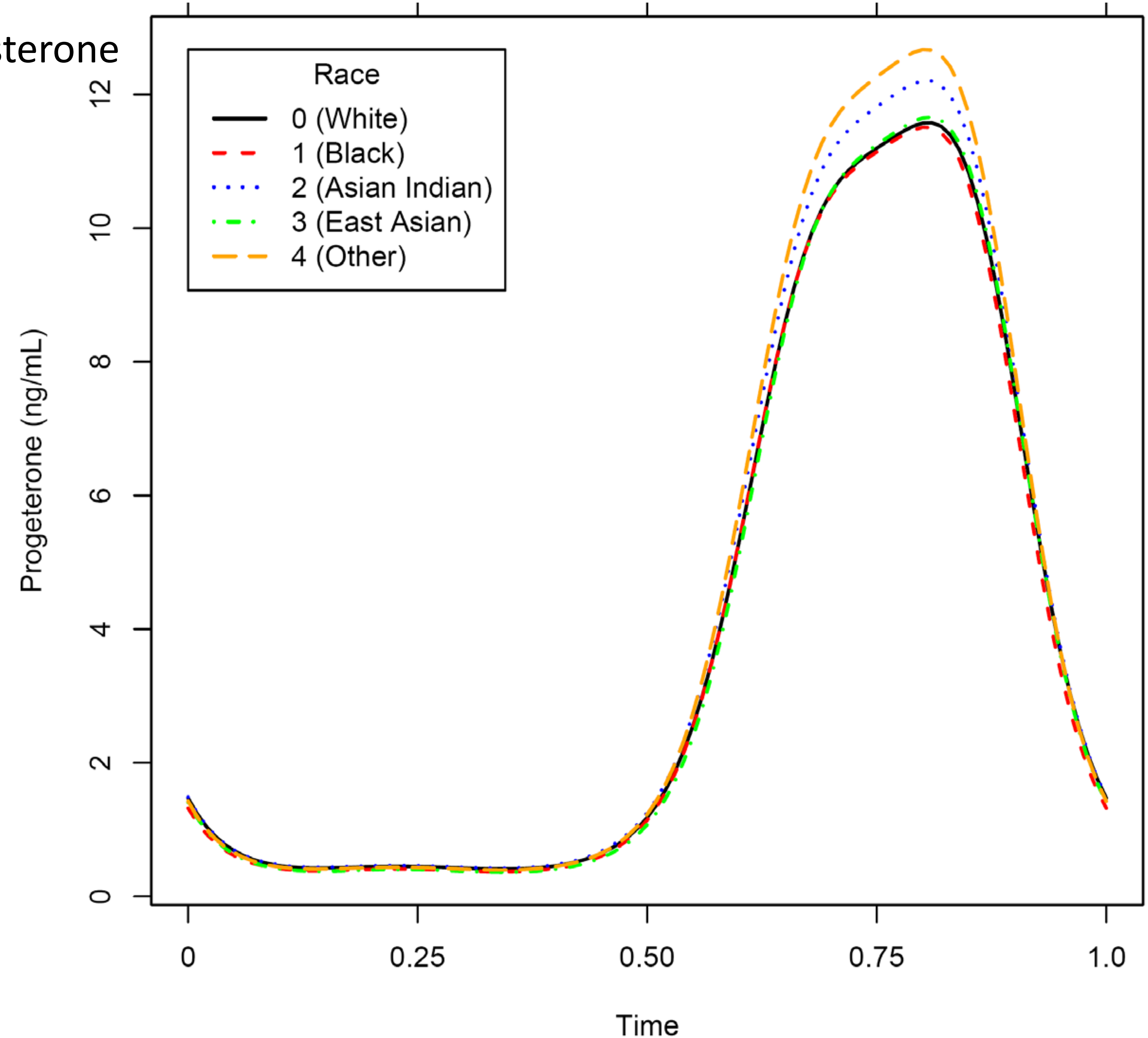


Race
0 (White)
1 (Black)
2 (Asian Indian)
3 (East Asian)
4 (Other)
LH (ng/mL)
5
10
15
20
0
0.25
0.50
0.75
1.0
Time

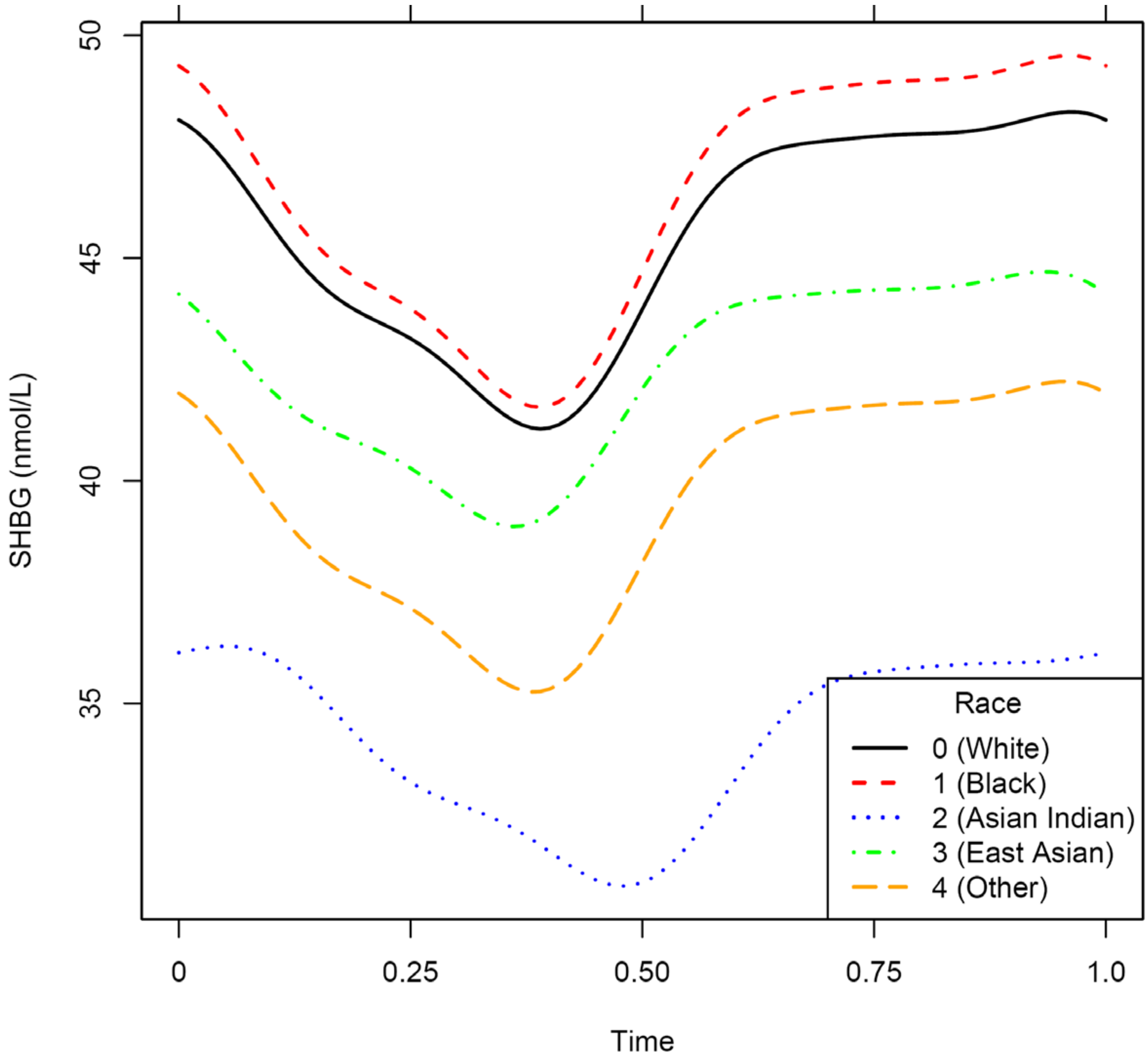
Mean (SE) LH levels by race

Race	n	Unadjusted	p-value	AGE + BMI	p-value
White	147	1.69 (0.03)		2.18 (0.15)	
Black	50	**-0.14 (0.06)**	**0.01**	**-0.17 (0.06)**	**<0.0001**
Asian Indian	11	-0.11 (0.10)	0.31	-0.15 (0.10)	0.15
East Asian	26	-0.04 (0.07)	0.57	-0.10 (0.07)	0.16
Other	14	0.005 (0.09)	0.96	-0.001 (0.09)	0.99

Progesterone



SHBG



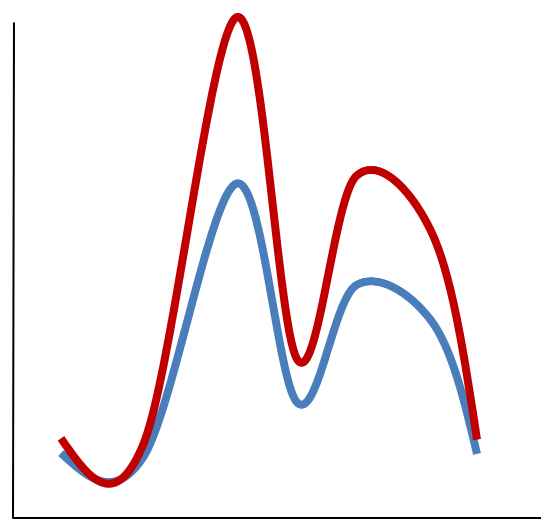
SHBG (nmol/L)
50
45
40
35
0
0.25
0.50
0.75
1.0
Time
Race
0 (White)
1 (Black)
2 (Asian Indian)
3 (East Asian)
4 (Other)

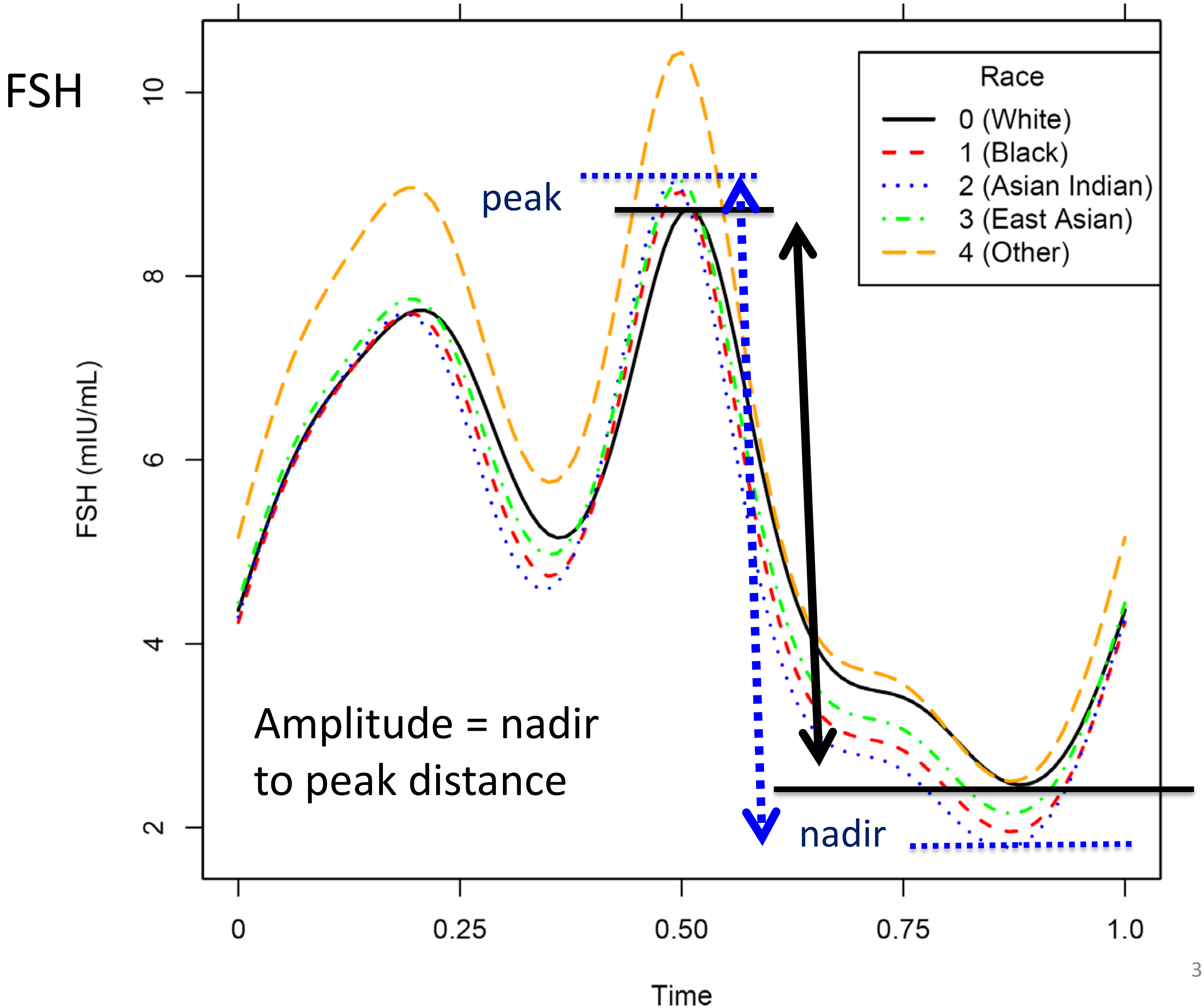
Nonlinear Mixed Models with Harmonic Terms

Albert and Hunsberger - Biometrics 2005

$$y_{ij} = \phi_{1i} + \exp(\phi_{2i})\, f\{t_{ij} - a\,\mathrm{logit}(\phi_{3i})\} + \varepsilon_{ij}$$

Amplitude: nadir to peak distance




FSH
peak
nadir
Amplitude = nadir to peak distance
Race
0 (White)
1 (Black)
2 (Asian Indian)
3 (East Asian)
4 (Other)
FSH (mIU/mL)
Time
0
0.25
0.50
0.75
1.0
2
4
6
8
10

Amplitude of FSH by race

Race	n	Unadjusted	p-value	AGE + BMI	p-value
White	147	0.57 (0.03)		reference	
Black	50	**0.17 (0.05)**	**0.0004**	**0.19 (0.05)**	**0.0001**
Asian Indian	11	**0.23 (0.09)**	**0.006**	**0.25 (0.08)**	**0.002**
East Asian	26	**0.13 (0.06)**	**0.03**	**0.19 (0.06)**	**0.002**
Other	14	0.124(0.08)	0.13	0.13 (0.08)	0.11

Summary of Results

	Mean		Amplitude	
E2	↑	Black + Asian Indian women	↑	Black women
FSH		-	↑	Black, Asian Indian, East Asian women
LH	↓	Black women		-
P		-	↑	Black women (sig after adjust for age & BMI)
SHBG	↓	Asian Indian (ns after adjust for age & BMI)		-

Decisions prior to analysis…

1. Time scale

2. Number of Harmonics

Modeling Time

- Actual visit days (calendar time)
- Scheduled visit days (biological time)

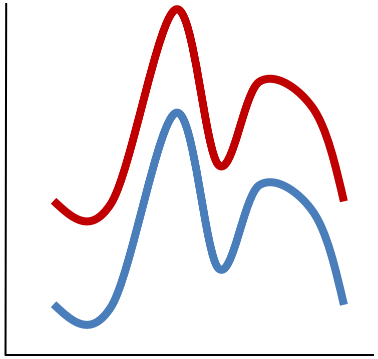
→ Linear mixed models

- Actual visit days standardized by cycle length
- Registered cycles (centered on ovulation)

Nonlinear mixed models with harmonic terms

Alternative models of time

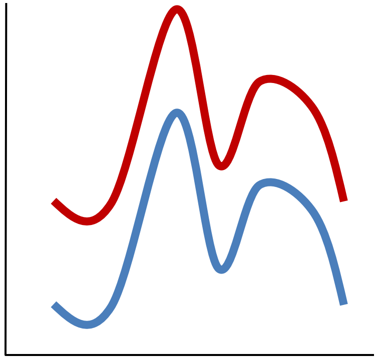
- How do the results using harmonic models compare with results from linear mixed models (SAS: *proc mixed*)?
- What happens to our results using the harmonic models when we do **not** center on ovulation at time of 0.5?



Mean Estradiol

	Harmonic model centered on ovulation	Linear Mixed model using biological days
White	4.31 (0.13)	4.62 (0.05)
Black	**0.21 (0.05)**	**0.26 (0.05)**
Asian Indian	**0.22 (0.09)**	**0.28 (0.10)**
East Asian	-0.04 (0.06)	-0.04 (0.07)
Other	0.10 (0.08)	0.11 (0.09)

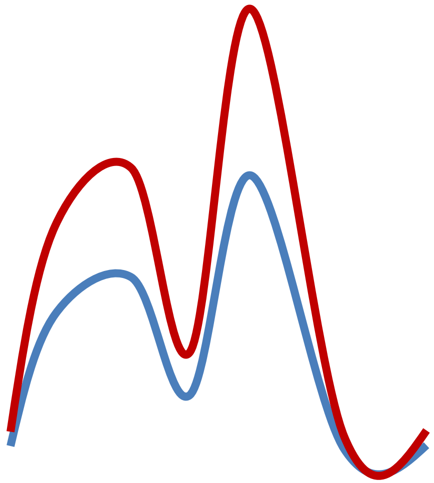
All estimates were adjusted for age and BMI.



Mean Estradiol

	Harmonic model centered on ovulation	Harmonic model NOT centered on ovulation	Linear Mixed model using biological days
White	4.31 (0.13)	4.27 (0.13)	4.62 (0.05)
Black	**0.21 (0.05)**	**0.21 (0.05)**	**0.26 (0.05)**
Asian Indian	**0.22 (0.09)**	**0.22 (0.09)**	**0.28 (0.10)**
East Asian	-0.04 (0.06)	-0.03 (0.06)	-0.04 (0.07)
Other	0.10 (0.08)	0.07(0.08)	0.11 (0.09)

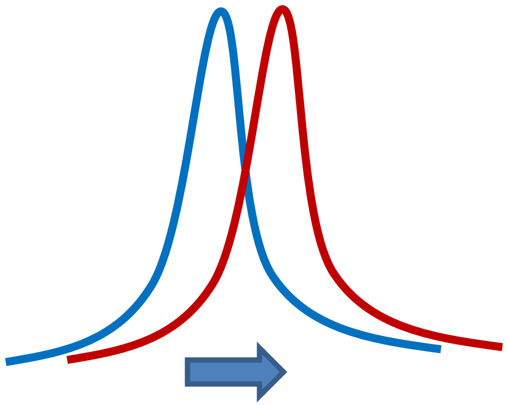
All estimates were adjusted for age and BMI.



Amplitude of FSH using different time scales

FSH	Centered Harmonic	Non-centered Harmonic
White (n=147)	ref	ref
Black (n=50)	**0.19 (0.05)**	**0.25 (0.05)**
Asian Indian (n=11)	**0.25 (0.08)**	**0.26 (0.09)**
East Asian (n=26)	**0.19 (0.06)**	**0.22 (0.07)**
Other (n=14)	0.13 (0.08)	0.16 (0.09)

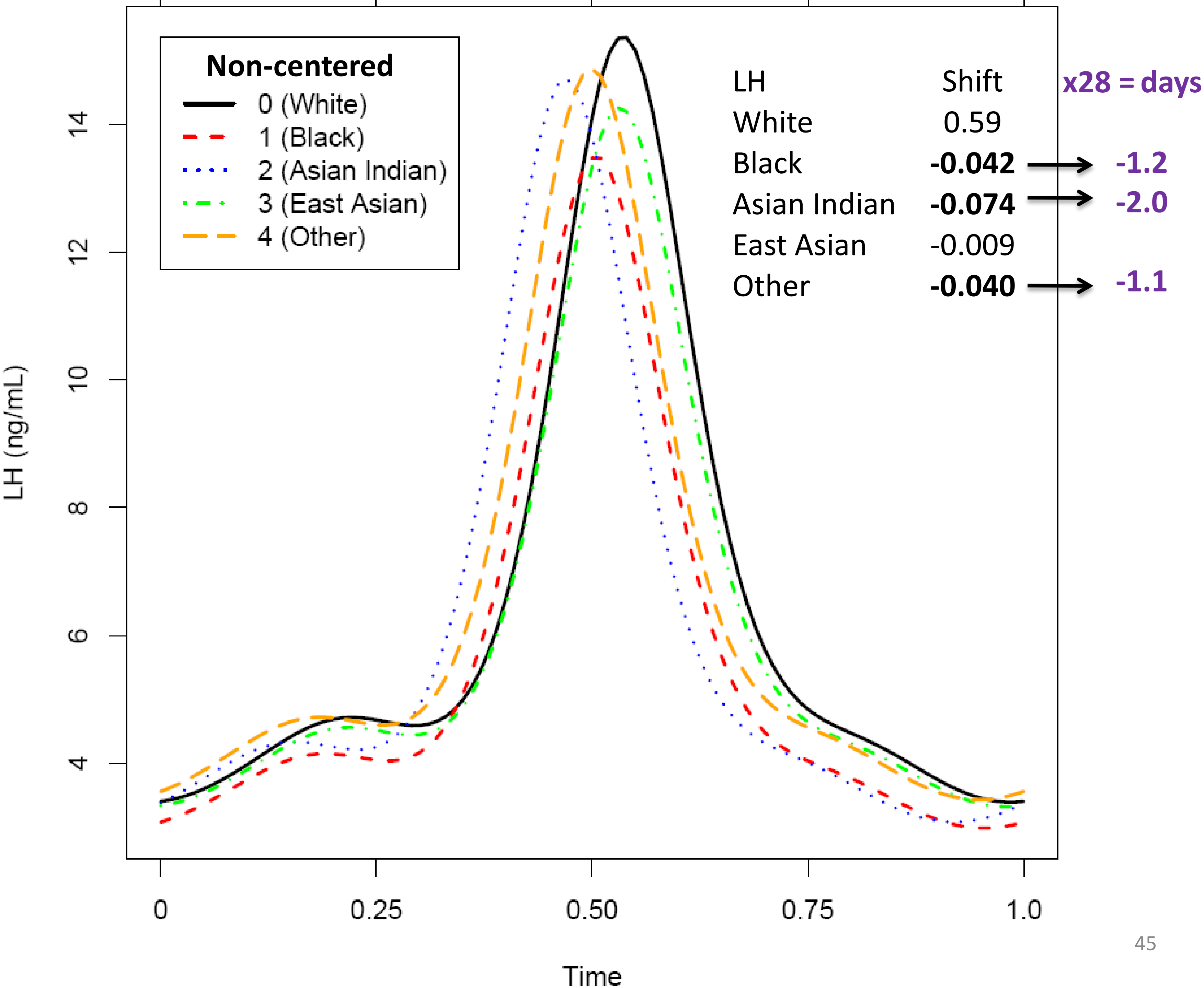
All estimates were adjusted for age and BMI.



Phase shift of LH

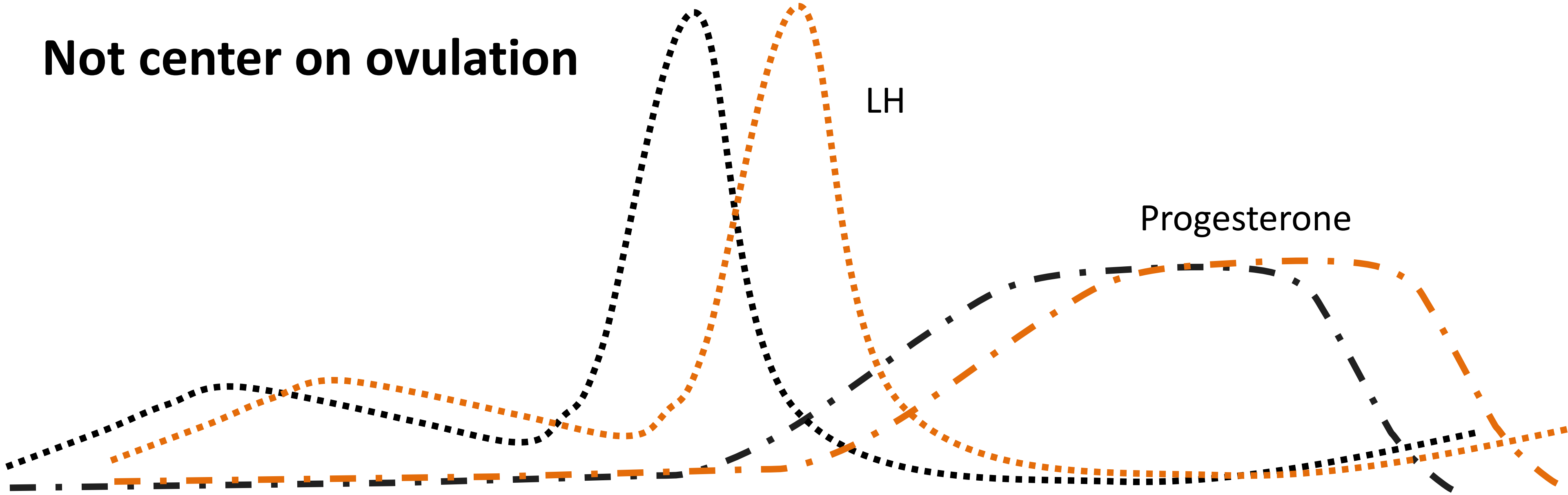
LH	Centered Harmonic	Non-centered Harmonic
White (n=147)	0.49	0.59
Black (n=50)	-0.010	**-0.042**
Asian Indian (n=11)	-0.006	**-0.074**
East Asian (n=26)	-0.008	-0.009
Other (n=14)	-0.005	**-0.040**

All estimates were adjusted for age and BMI.

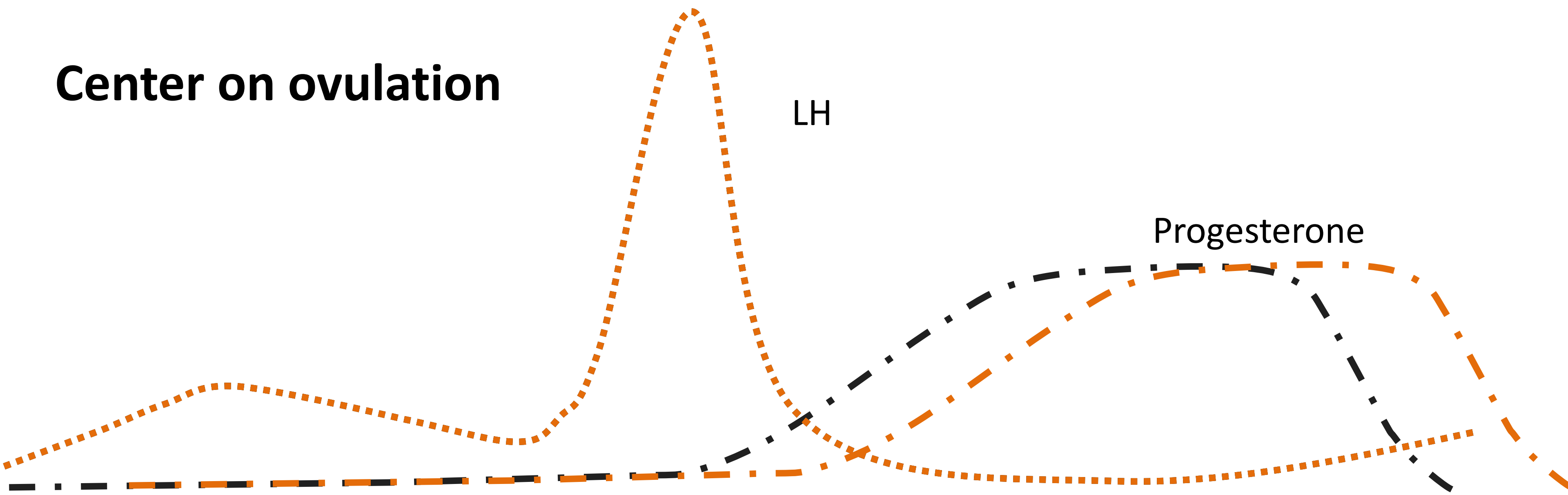

Non-centered
0 (White)
1 (Black)
2 (Asian Indian)
3 (East Asian)
4 (Other)
LH (ng/mL)
4
6
8
10
12
14
0
0.25
0.50
0.75
1.0
Time
LH Shift
White 0.59
Black -0.042 → -1.2
Asian Indian -0.074 → -2.0
East Asian -0.009
Other -0.040 → -1.1
x28 = days

What is the difference in inference between modeling time centered on ovulation and not centering on ovulation?

Not center on ovulation



Center on ovulation



Summary on alternative models of time

1. Linear mixed models
   – Similar findings in mean level as harmonic models
   – Do not easily measure amplitude and phase shift
2. Harmonic models centering or not centering on ovulation
   – Similar results for mean level and amplitude
   – Different results for phase shift
   – Decision for centering on ovulation or not depends on biological question of interest

Decisions prior to analysis…

1. Time scale

2. Number of Harmonics

Results for **mean E2** with different number of harmonics

Race	1	2	3	4	5
White	ref	ref	ref	ref	ref
Black	**0.175 (0.05)**	**0.179 (0.05)**	**0.187 (0.048)**	**0.188 (0.05)**	**0.188 (0.049)**
Asian Indian	0.173 (0.09)	**0.186 (0.09)**	**0.187 (0.091)**	**0.193 (0.09)**	**0.201 (0.093)**
East Asian	-0.012 (0.06)	-0.040 (0.06)	-0.041 (0.062)	-0.052 (0.06)	-0.049 (0.064)
Other	0.035 (0.08)	0.054 (0.08)	0.072 (0.080)	0.084 (0.08)	0.082 (0.083)

Results for **amplitude FSH** with different number of harmonics

Race	1	2	3	4	5
White	ref	ref	ref	ref	ref
Black	**0.261 (0.06)**	**0.195 (0.04)**	**0.175 (0.049)**	**0.170 (0.05)**	**0.157 (0.049)**
Asian Indian	**0.366 (0.10)**	**0.228 (0.06)**	**0.228 (0.087)**	**0.232 (0.08)**	**0.220 (0.088)**
East Asian	**0.228 (0.08)**	**0.135 (0.05)**	**0.131 (0.063)**	**0.130 (0.06)**	0.102 (0.062)
Other	0.160 (0.11)	0.129 (0.07)	0.113 (0.083)	0.124 (0.08)	0.111 (0.082)

Conclusion

- Factors that determine race may affect sex hormone regulation
- Modeling menstrual cycle patterns with harmonic models has advantages
  - Not necessary to have uniform visits
  - Easily estimates amplitude and phase shift
  - Model time in different ways depending on research question of interest
  - Account for confounding on all three aspects of the hormonal pattern

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- Site PI: Jean Wactawski-Wende
- BioCycle working group: Sunni Mumford, Anna Pollack, Aijun Ye, Cuilin Zhang
- Participants of the BioCycle Study

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