Changes with age in the circadian rhythm of circulating melatonin

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Background: Effects of Melatonin in the body

- Melatonin is implicated in modulation of circadian rhythms, acting as a synchronizer of hormones and systems throughout the body, including the sleep/wake cycle.
- Circulating melatonin peaks as you become drowsy, and drops when you awaken.
- Blue light, a large component of daylight sunlight on earth, is believed to be a synchronizer for melatonin. Melatonin is reduced upon exposure to blue light. Therefore electronics and some types of LED lighting can interfere with normal cycles, especially evening exposure . Circadian disruption can have negative health effects.
- Melatonin is the most powerful free-radical scavenger and anti-oxidant. It also acts to enhance the effect of other anti-oxidants. Unlike other radical scavengers, its metabolites are also anti-oxidants. It is considered twice as effective as vitamin E.
- Melatonin interacts with the immune system, creating an anti-inflammatory effect

Background: aging, hormones, and circadian rhythm changes

We're looking at Melatonin to see how it changes with age.

A number of variables are known to decrease in circadian amplitude as we age, and advance in circadian phase:

- Prolactin, estrogens (E1 and E2), 17-OH-progesterone, aldosterone, DHEA-S:
  - The circadian amplitude is statistically significantly reduced in post-menopausal women as compared to adult menstruating women.
- Blood pressure and heart rate:
  - We demonstrated in several populations that the circadian amplitude decreased and the acrophase advanced in older people.

Background: aging, hormones, & circadian amplitude changes

Two-way ANOVA: 7 plasma hormones have a significant decrease in amplitude in post-menopausal women (Age III).

Hormone (units)	N	Age I	Age II	Age III
Prolactin (ng/ml)	29	12.4	16.5	11
E1 (pg/ml)	27	17.6	15.9	11.4
E2 (pg/ml)	26	28	28.4	8.1
17-OH Progesterone (pg/ml)	29	181	196	128
Aldosterone (ng/dl)	25	4.1	2.5	1.8
DHEA-S (ng/ml)	28	580	370	230

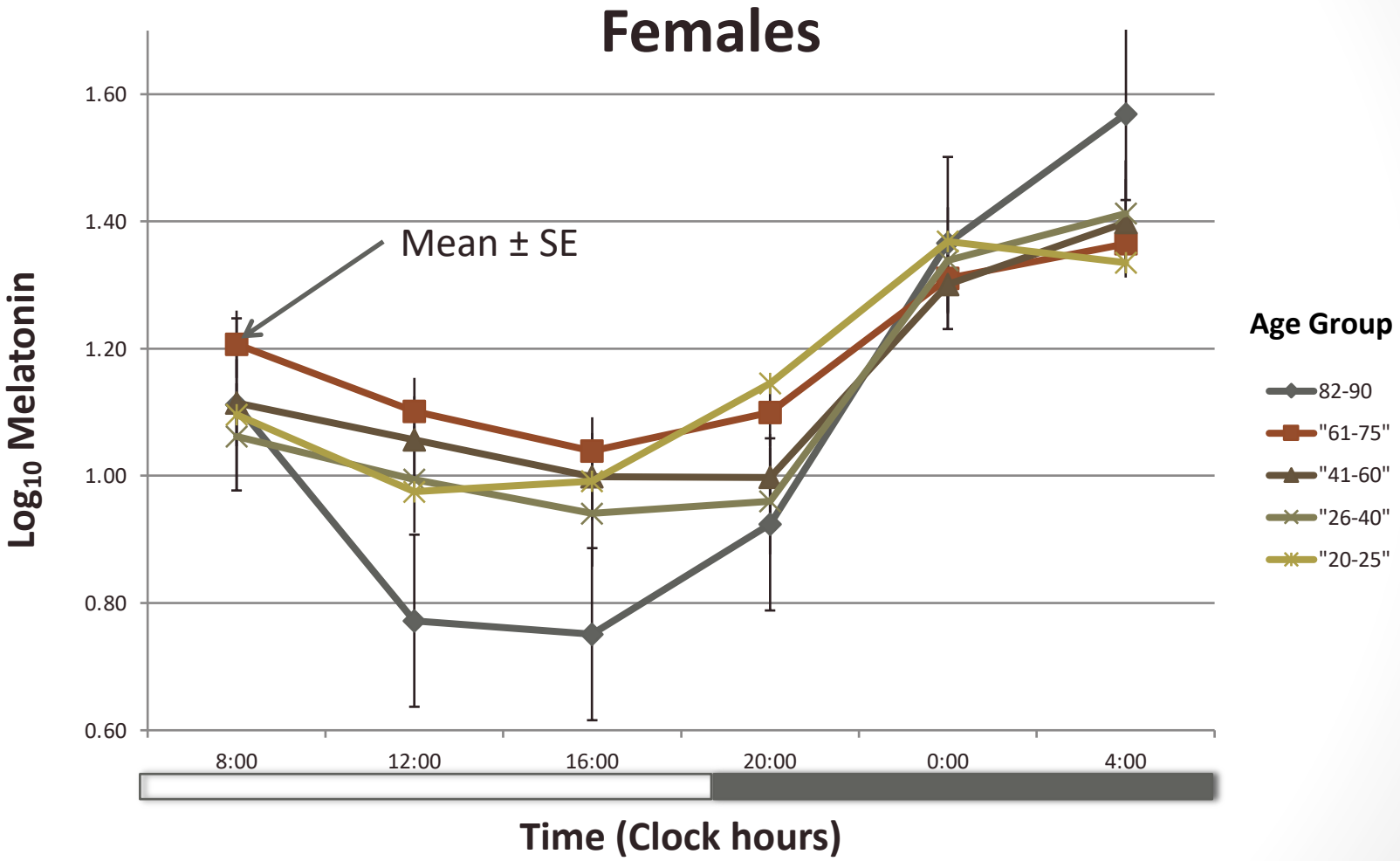
Subjects & Methods

- Two separate studies, collecting circulating melatonin every 4 hours for a full day [*Prof. Brunetto Tarquini*]
  - Study A: 133 Females; 40 males; (173)
  - Study B: 111 Females; 61 males; (172)
  - Mostly clinically healthy subjects
  - Florence, Italy
  - Ages: 20-90
  - Mean ± SD age: 48.5 ± 17.1
- Circulating melatonin was determined by radioimmunoassay
- Sensitivity 3 pg/ml
- Intra- and inter-assay variability were 6.6% and 5.9% respectively

Analyses: log Melatonin vs Age

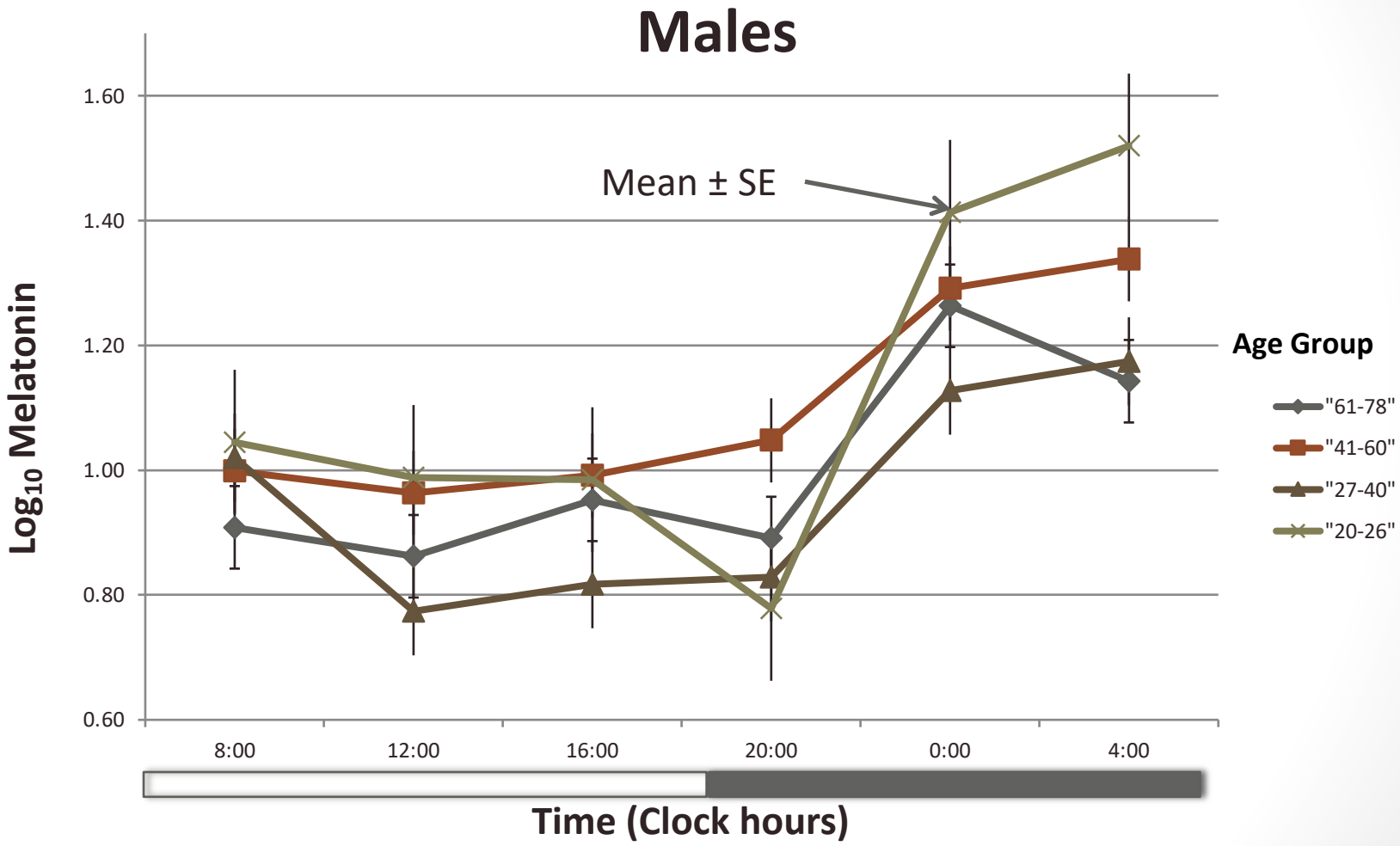
- ANOVA of log data vs age, 20-90
  - by gender broken into 5 different age groups
  - Study A only
- 24-hr Cosinor of log data: amplitude, MESOR, acrophase
- Regression of MESOR, Amplitude vs age, by gender
  - MESOR versus age (linear), by gender
  - Amplitude versus age (linear), by gender
  - MESOR versus age (quadratic), by gender
  - Amplitude versus age (quadratic), by gender
  - on studies A and B separately, and together
- Population Mean Cosinor of 24-hr Cosinor results
  - by gender broken into 5 different age groups
  - Results in an average of MESOR, and a vectorial average of circadian amplitude and acrophase
- Parameter tests comparing circadian rhythm among age groups

ANOVA: log Melatonin vs Age



ANOVA P-values <.05 for all age groups. Note the large amplitude in ages 82-90.

ANOVA: log Melatonin vs Age

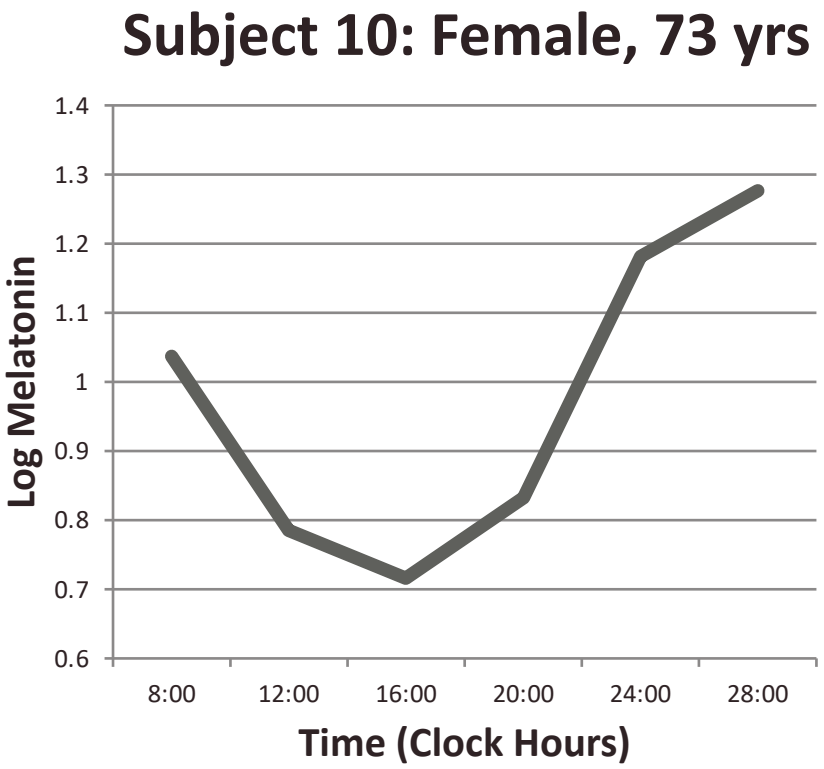


ANOVA P-values <.05 for all age groups.

Cosinor: 24-hr data collection

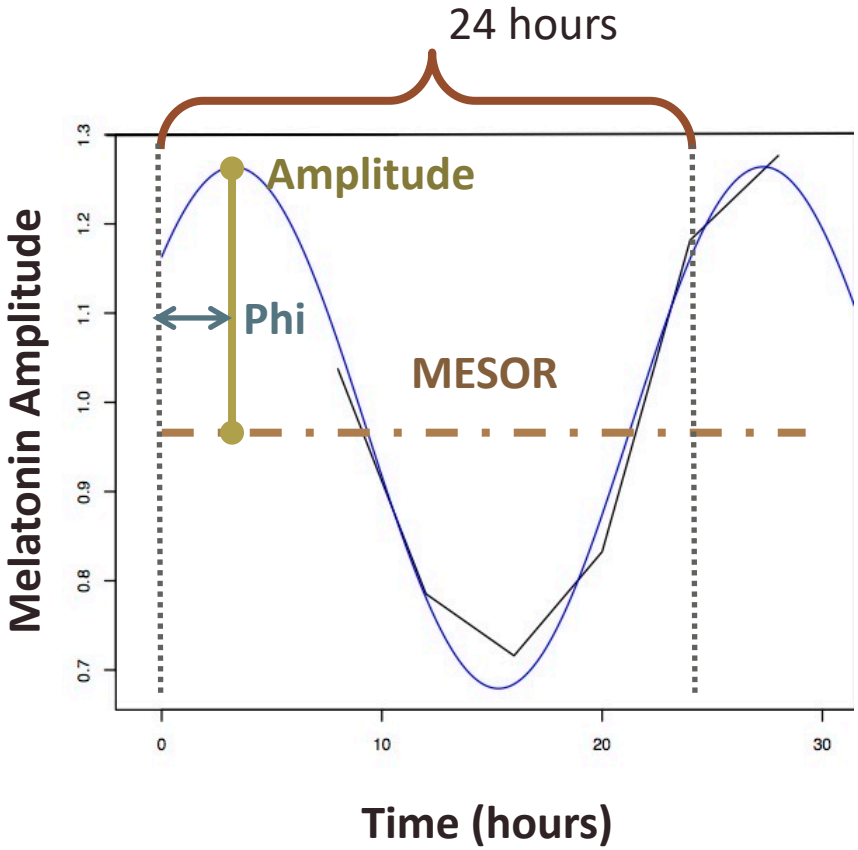
Log Data

ID	10
Gender	F
Age	73
Date	19940202
time1	8:00
Mel1log	1.0374
time2	12:00
Mel2log	0.78533
time3	16:00
Mel3log	0.716
time4	20:00
Mel4	0.83251
time5	24:00
Mel5	1.1818
time6	28:00
Mel6	1.2765



Cosinor performed individually on all 345 study subjects

Cosinor: 24-hr trial period

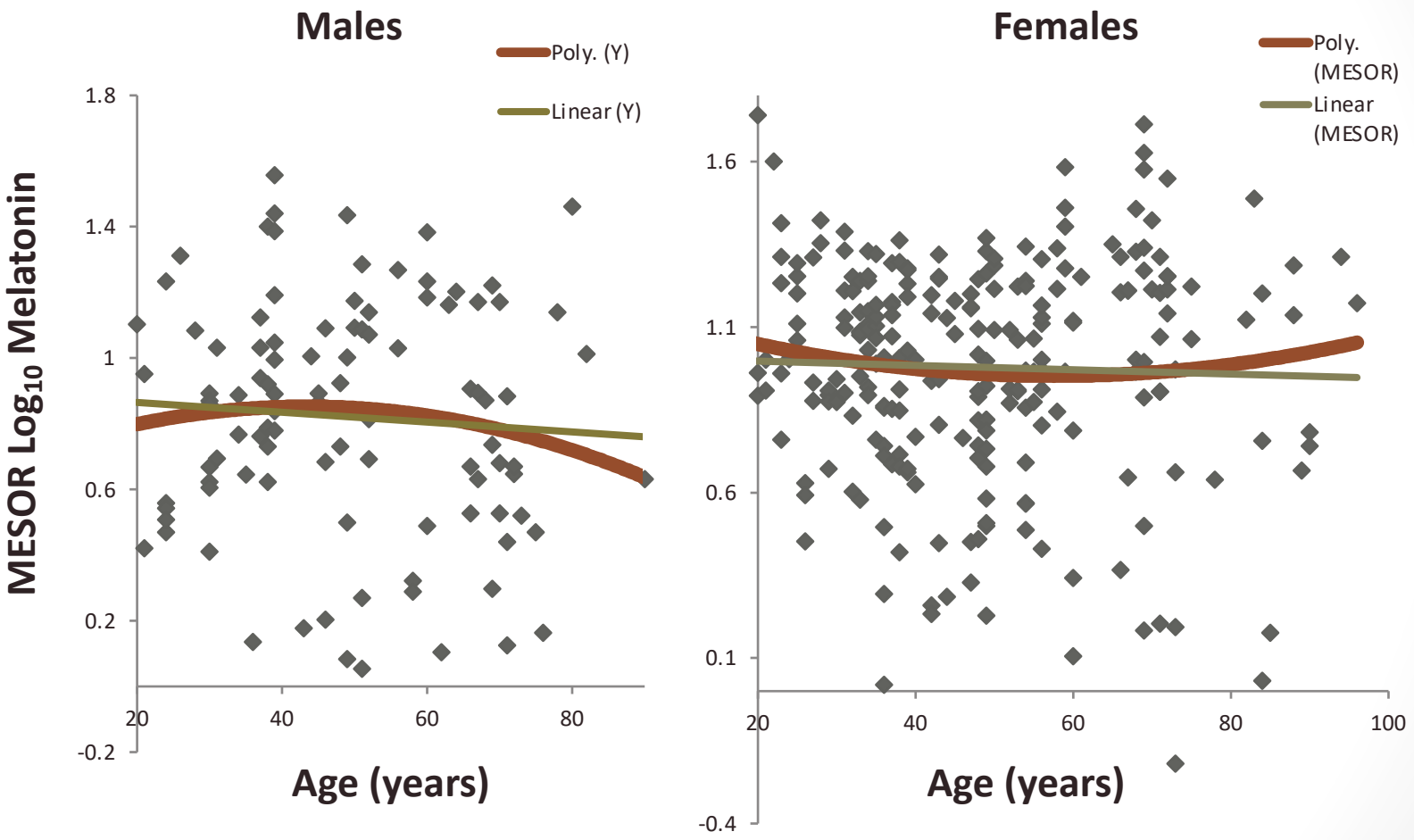


Subject 10:
Cosinor Results

ID	10
PR	98.0
MESOR	0.972
24hA	0.292
24hPhi	-49.0

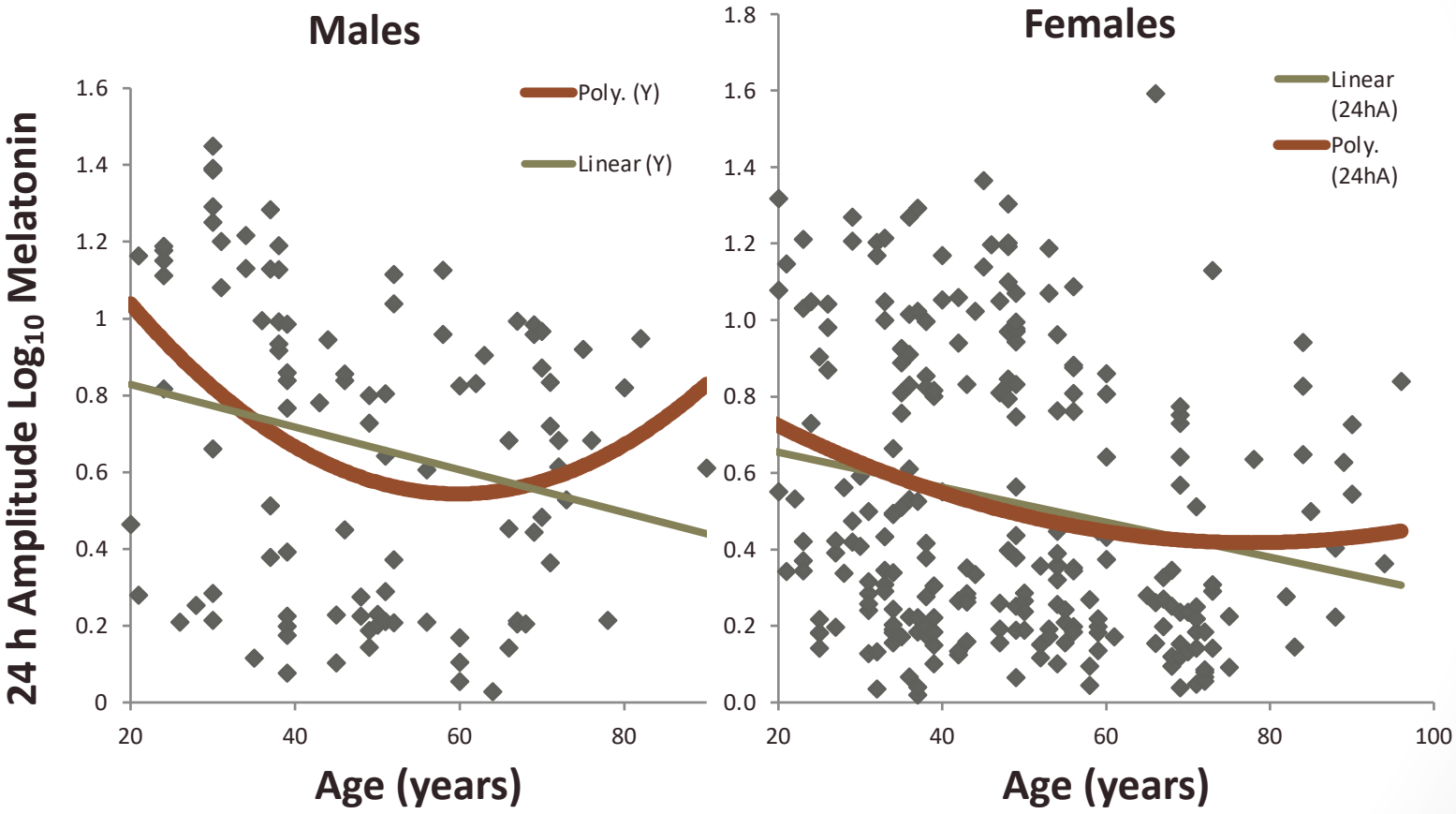
Cosinor performed individually on all 345 study subjects

Regression: MESOR vs age



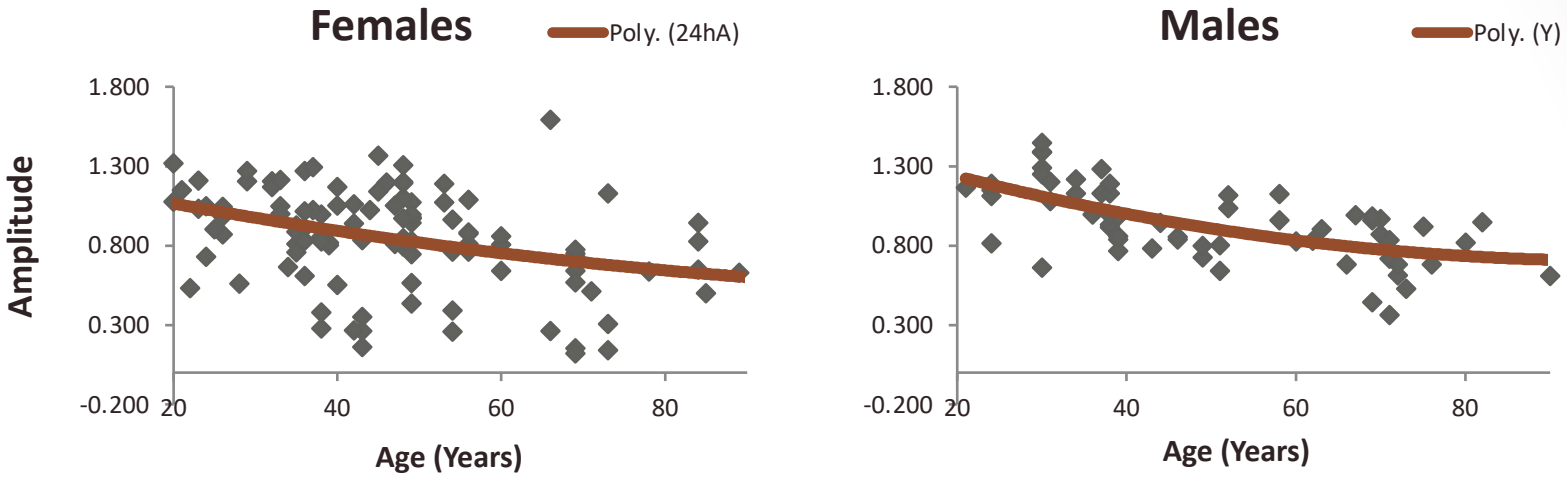
The slope of the regression line is not significantly different from 0 for either gender.

Regression: Amplitude vs age

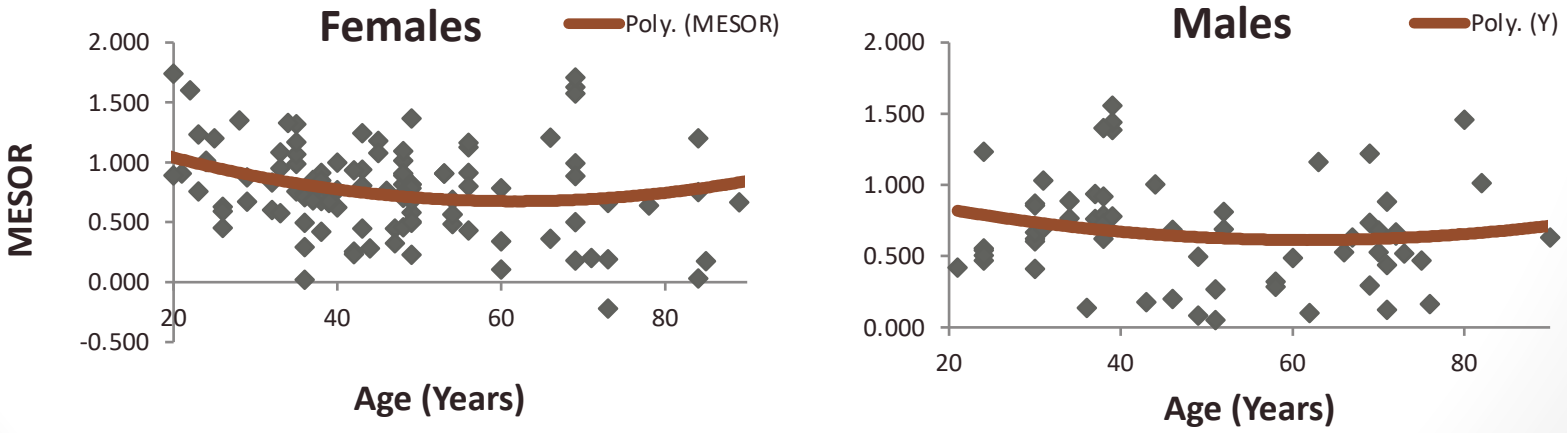


The slope of the regression line is significantly different from 0 for both genders.

Regression: Study B



The slope of the regression line is significant for both genders (P<.001).



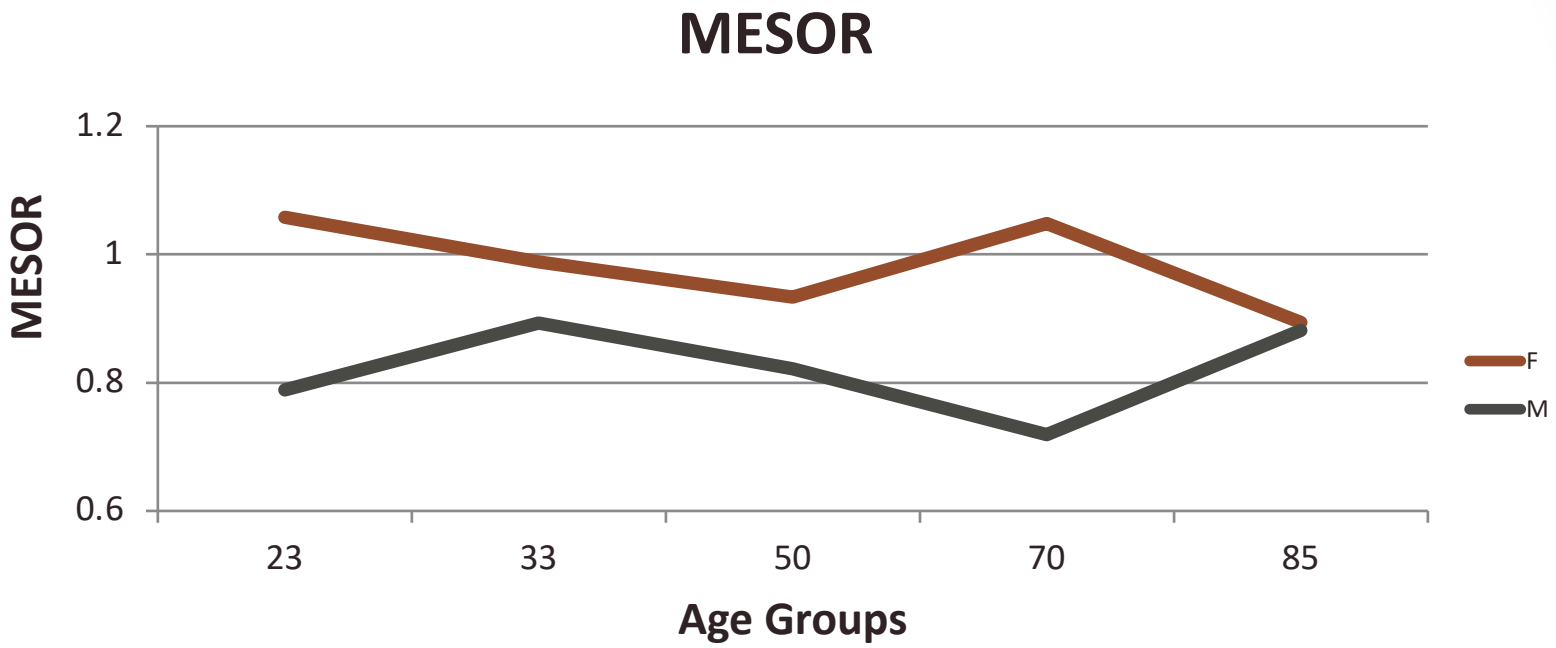
The slope of the regression line is significant for females (P=.02), but not males (P=.49).

Population Mean Cosinor

- Population mean cosinor assesses the presence of a rhythm on a population basis
- It averages rhythm characteristics from individual subjects
- All age and gender groups are sigificant except largest and smallest in males.

Ages	Female P-value	Male P-value
<26y	**<0.001**	0.003
27-40y	**<0.001**	**<0.001**
41-60y	**<0.001**	**<0.001**
61-75y	**<0.001**	**<0.001**
>75y)	**<0.001**	0.106

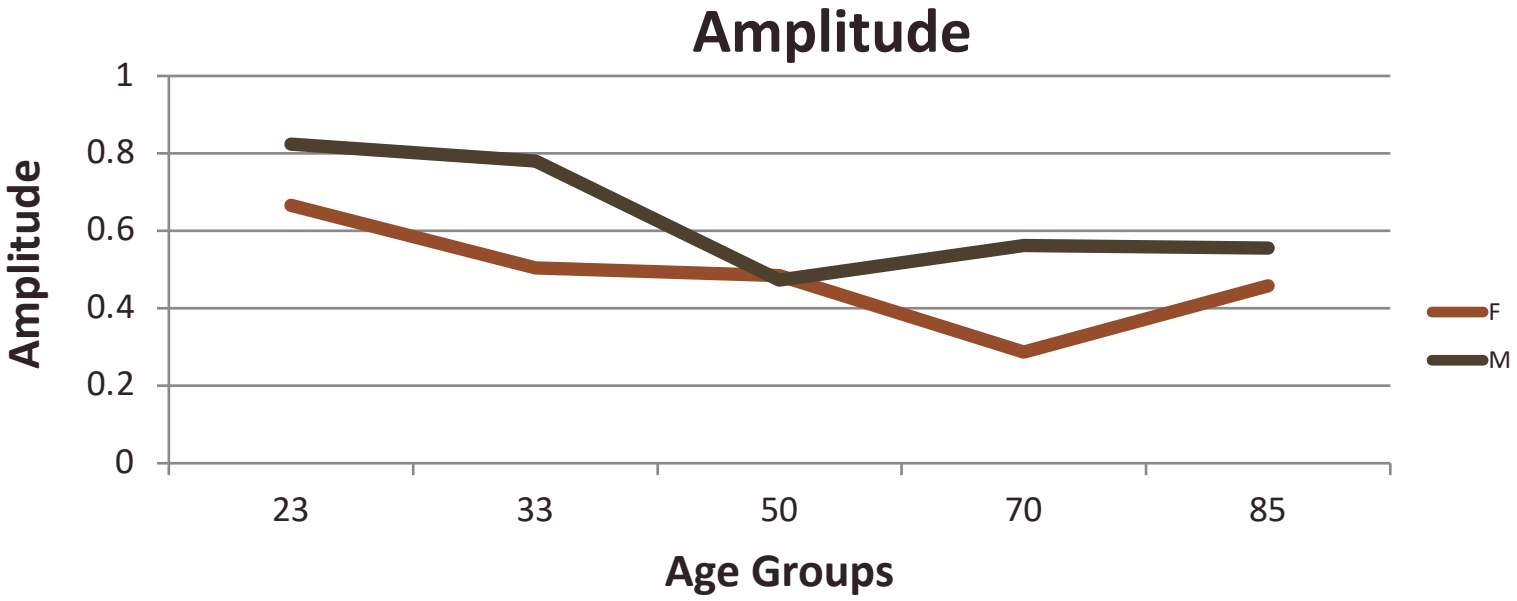
Population Mean Cosinor Parameter Test for F & M



Parameter Test comparing MESOR for M or F across age groups

- There is no significant change of log Melatonin concentrations across ages, for either gender (M P=.49; F P=.24).
- Females have a significantly higher MESOR than males. (P=.0001)

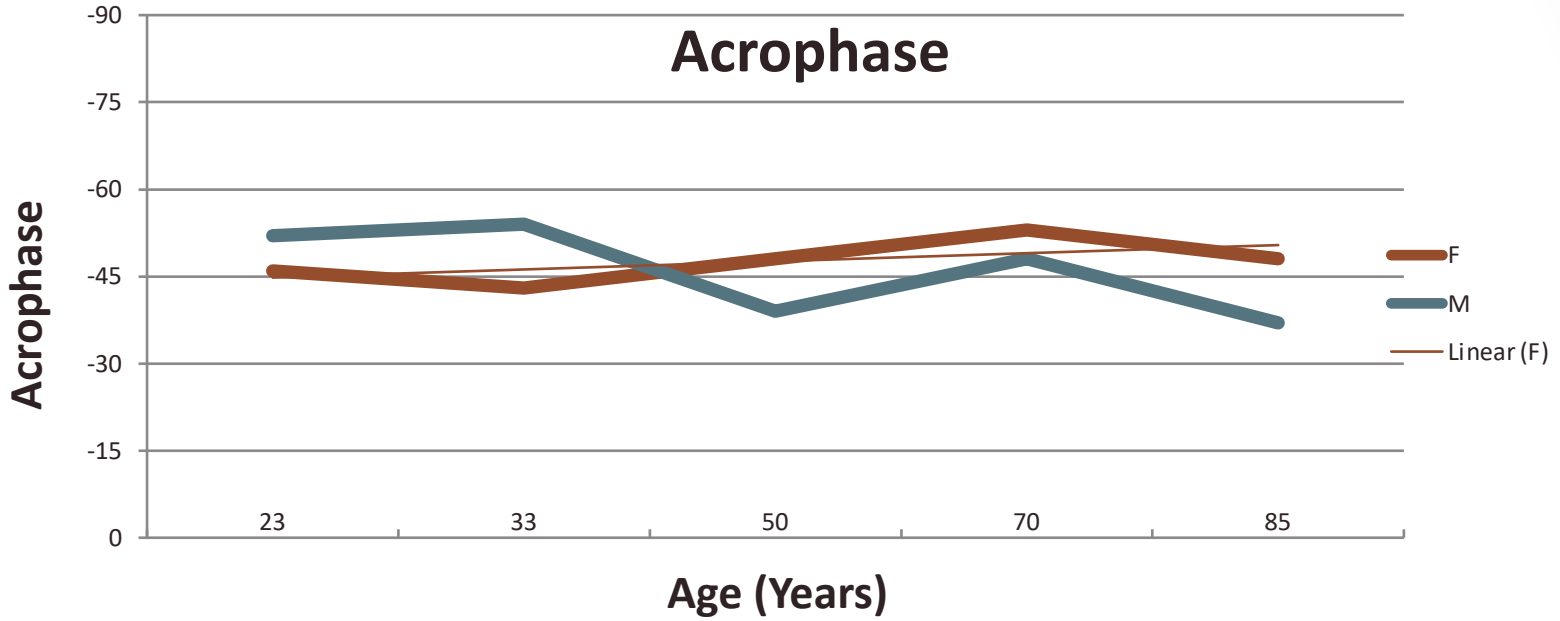
Population Mean Cosinor Parameter Test for F & M



Parameter Test comparing Amplitude for M or F across age groups

- There is a significant drop of log Melatonin amplitudes across ages, for both genders (M P=.015; F P=.0033).
- Males have a significantly higher amplitude than Females (P=.001).

Population Mean Cosinor Parameter Test for F & M



Parameter Test comparing Acrophase for M or F

- There is a significant advance of log Melatonin acrophase across ages for males, but no significant change for females (M P=.039; F P=.594) .**Males**: (-52° → -39°) ~52 min earlier; **Females**: (-46° → 48°)
- There is no significant difference between male and female acrophases

Summary:

- Amplitudes of log melatonin fall with age in both women and men
- There is a plateau, or slight uptick in amplitude in the oldest women and men
- The circadian amplitude of log melatonin is higher in men than in women
- The MESOR of log melatonin is higher in women than in men
- Acrophase shifts earlier by approx 1 hour in males (P=.038) but not in females.

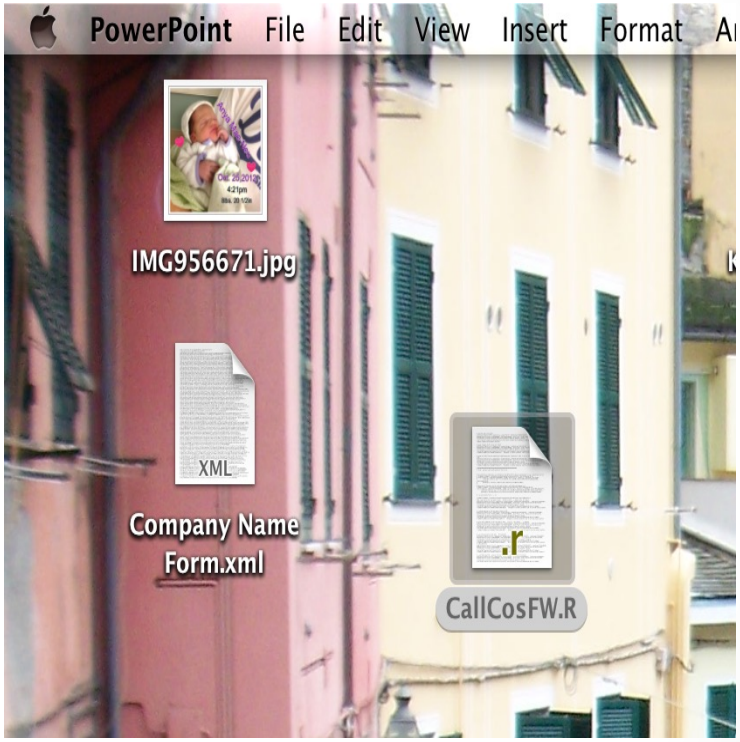
Conclusions

- **Decreasing amplitude and a phase advance with increasing age** is found in numerous studies on diverse variables.
- Less well known is the possible **plateauing or even rebound** we saw here in the very old.
- This **rebound is also observed in several endpoints of heart rate variability**, including: RR50, SDmean, and HF power.
- It is possible that the **oldest group were the healthier individuals**, in view of the old age they reached. Further study is needed. The uptick raises the question of whether **studies should carefully adjudicate the state of health or disease** in elderly subjects and whether to include those who are not clinically healthy.
- Longitudinal studies allow identification of predisease, adding another level of assessment of health. (Those who develop disease can be retroactively removed from the study cohort.)
- The trend in melatonin amplitudes with age support a **need for age- and gender-appropriate reference data** that can provide a more refined understanding of what constitutes “healthy” levels of melatonin

CATkit: Cosinor Analysis Toolkit

- Install R
- z.umn.edu/CATkit
- .r script on the desktop
- Double click to run

Slight changes in the script are made for each new technique



Thank you!

Halberg Chronobiology Center

CATkit: z.umn.edu/CATkit