Circadian rhythm in health and disease

Satchin Panda
Salk Institute
satchin@salk.edu
panda@salk.edu

Megumi Hatori (Keio U)
Christopher Vollmers (UCSC)
Luciano DiTachhio (KUMC)
Amir Zarrinpar (UCSD)
Shubhroz Gill (Broad inst)

Collaborator
Girish Melkani (SDSU)

Salk Innovation Fund
AFAR
Helmsley Foundation

Post-Doc fellowships
ADA
AASLD
Helmsley Foundation
Glenn Foundation

ILSI North America FNSP Mid-year meeting, 18 July, 2017
1900

- Life expectancy at birth: 47 years
- 1 in 100 lived beyond 90 years of age.
- 1/3rd of all newborn died before age 5.
- Leading causes of death were infectious diseases.
- **Microbial (foreign agent) cause of disease.**
- Sanitation, vaccines, and antibiotics saved lives.

2010

- Life expectancy at birth: 79 years
- 1 in 4 may live beyond 90 years of age.
- 1/3rd of all adults suffer from at least one non-infectious chronic disease. 80% of elders >65y age have multiple chronic diseases that require treatment for 1 y or longer.
- **Lifestyle contributes to disease risk.**
- Cure is rare; current treatments help people live with disease and disability.
Lifestyle is what, **WHEN** and **how much** we **Eat, Sleep, and Move** on a daily basis.
Deep sleep

Body temperature rises

Melatonin drops

Corticosteroids rise

High alertness

Muscle performance peaks

Body cools down

Melatonin rises

Bowel movement likely
Daily rhythms in physiology, metabolism, and behavior
Alertness

Sleep

Few thousand Blue light sensing neurons in each eye tune our brain clock to light.

Orange glow from fire or candle light does not stimulate melanopsin.

Sleep hormone melatonin rises and supports sound sleep.

Bright day light stimulates melanopsin.

Synchronizes brain clock

Raises alertness

Reduces depression
Light for vision is not light for health

**Bright indoor nights**

- Blue-rich LEDs or even an hour of dim blue light from phone screens and tablets activates melanopsin.
- Disrupts circadian rhythms
- Reduces sleep hormone melatonin.
- Keeps us awake.

**Gloomy indoor days.**

- Misaligns circadian rhythm from day:night cycle
- Reduces alertness
- Promotes depression and mood disorders
- Reduces performance
Daily rhythms in physiology, metabolism, and behavior
Genome-wide transcriptional rhythms in liver of ad lib fed mice

~ 3000 probesets show 24 h rhythms.
Circadian rhythm may temporally optimize metabolism

Temporal separation of incompatible processes
(anabolic and catabolic pathways)

Synchronized oscillations of a given pathway
Healthy (low fat mass, normal blood sugar, cholesterol, Insulin, leptin)

Standard Diet

Obese & Diabetic (increased fat mass, high blood sugar, cholesterol, Insulin, leptin)

High-fat diet
Burning carb and storing fat

Burning fat
Burning carb and storing fat

6am
Noon
6pm
Midnight

Burning fat
Burning carb and storing fat

Burning fat
Eat 10 h

Eat 15 h
Fatty Liver

Healthy Liver

Body weight

28%

Body fat

70%

Weeks

Body weight

Week 28%

Week 70%
Results: Food Intake

Results: Activity

Liver Metabolites

Normal chow  High Fat Diet

Dark/Light  Food access

NA  NT  FA  FT

Total named metabolites

324

Changed between any pair of feeding regimens
(P < 0.05, n = 8)

240

Changed between HF and NC diet

232

Sugar and nucleotide metabolism

324

Fatty acid metabolism

Hatori et al. Cell Metab. 2012
Sugar and Nucleotide metabolism

Normal chow  High Fat Diet

Hatori et al. Cell Metab. 2012
Fatty acids and bile metabolism

Normal chow

High Fat Diet

Fatty acid metabolism

Hatori et al. Cell Metab. 2012
Liver metabolism is strongly circadian. Diurnal rhythms impact key nodes and metabolites of central metabolic pathways.
Hatori et al. Cell Metabolism 2012
Time Restricted Feeding (TRF)

Time of food access is restricted
No caloric or nutrient restriction
TRF can prevent obesity and metabolic diseases in mice fed a High Fat Diet

What are the temporal boundaries of TRF? Is 8 h a magic number?
Is TRF effective against other nutrients (high sucrose, high fructose)?
Is TRF therapeutic on pre-existing obesity?
Does TRF leave a legacy effect after the animals are released to ad lib feeding?
Food intake

A. Cumulative Food Consumption

(i) 

(ii) 

F. Daily caloric consumption in the 5T2A cohort

5 days TRF  2 days ALF

Day 1 Day 2 Day 3 Day 4 Day 5 Day 6 Day 7

9h TFR

15h (remainder of the day)
TRF is preventative and therapeutic

A. Body Weight

B. Body Composition
TRF preserves adipose tissue function

A. eWAT and BAT H&E Staining

<table>
<thead>
<tr>
<th>eWAT</th>
<th>FSA</th>
<th>FST</th>
<th>FA</th>
<th>9hFT</th>
<th>5T2A</th>
<th>FAA</th>
<th>FTT</th>
<th>FTA</th>
<th>FAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAT</td>
<td></td>
<td></td>
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</table>

300μm
Eating pattern and serum cholesterol

A. Serum Cholesterol Concentration

(i) Serum Cholesterol (mg/dL)

<table>
<thead>
<tr>
<th></th>
<th>FSA</th>
<th>FST</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>150</td>
<td>170</td>
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</table>

(ii) Serum Cholesterol (mg/dL)

<table>
<thead>
<tr>
<th></th>
<th>FA</th>
<th>9hFT</th>
<th>12hFT</th>
<th>5T2A</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>200</td>
<td>180</td>
<td>150</td>
<td>220</td>
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</table>

(iii) Serum Cholesterol (mg/dL)

<table>
<thead>
<tr>
<th></th>
<th>NA</th>
<th>15hNT</th>
<th>FA</th>
<th>15hFT</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>120</td>
<td>250</td>
<td>180</td>
</tr>
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</table>

(iv) Serum Cholesterol (mg/dL)

<table>
<thead>
<tr>
<th></th>
<th>FAA</th>
<th>FTT</th>
<th>FTA</th>
<th>FAT</th>
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<tbody>
<tr>
<td>0</td>
<td>200</td>
<td>100</td>
<td>150</td>
<td>120</td>
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</tbody>
</table>

* ** *** ns ***
Hepatic and serum triglycerides

D. Hepatic Triglyceride Content

(i) *** ns
(ii) ** ns *
(iii) *** * ns
(iv) ** * ns

E. Serum Triglyceride Concentration

(i) ns
(ii) ** ****
(iii) ns
(iv) ns
TRF effects on muscle performance

A. Rotarod PeA. Grip Strength of the Forelimb Muscles
Time Restricted Feeding (TRF)

Time of food access is restricted
No caloric or nutrient restriction
TRF can prevent obesity and metabolic diseases in mice fed a High Fat Diet

What are the temporal boundaries of TRF? Is 8 h a magic number?
Is TRF effective against other nutrients (high sucrose, high fructose)?
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Food intake

A. Cumulative Food Consumption

(i) Cumulative kcal vs. weeks for FSA and FST

(ii) Cumulative kcal vs. weeks for various conditions

F. Daily caloric consumption in the 5T2A cohort

- 5 days TRF
- 2 days ALF

Bar graph showing kcal/mouse/day for different days and conditions:
- Day 1 to Day 7
- 9h TFR
- 15h (remainder of the day)
TRF is preventative and therapeutic
Short term (13+12) cross-over
TRF preserves adipose tissue function

A. eWAT and BAT H&E Staining

<table>
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300µm
Eating pattern and serum cholesterol

A. Serum Cholesterol Concentration

(i) - (iv) show graphs comparing different eating patterns and their effects on serum cholesterol levels. Each graph represents a different group or condition, with bars indicating cholesterol levels and error bars showing variability. Statistical significance is indicated by symbols such as *, **, and *** for p-values.
Hepatic and serum triglycerides

D. Hepatic Triglyceride Content
(i) (ii) (iii) (iv)

E. Serum Triglyceride Concentration
(i) (ii) (iii) (iv)
TRF effects on muscle performance

A. Rotarod PeA. Grip Strength of the Forelimb Muscles
Summary-II

TRF is preventative and therapeutic against obesity and metabolic diseases.

TRF exerts pleiotropic effects on multiple organs (liver, muscle, WAT, BAT).

Occasional ad lib feeding can be counterbalanced by TRF, however, TRF does not exert profound legacy effects.
Food  Food  ALF

Food  No Food  TRF

**Daily Activity**

<table>
<thead>
<tr>
<th></th>
<th>ALF</th>
<th>TRF</th>
<th>ns</th>
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</thead>
<tbody>
<tr>
<td><strong>Day</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Night</strong></td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Body weight (mg)**

<table>
<thead>
<tr>
<th></th>
<th>ALF</th>
<th>TRF</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Night</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
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</table>

**Daily Sleep**

<table>
<thead>
<tr>
<th></th>
<th>ALF</th>
<th>TRF</th>
<th></th>
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<tbody>
<tr>
<td><strong>Day</strong></td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td><strong>Night</strong></td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>
Radial contractility
Fractional shortening (FS) = (DD - SD) / DD

Heartbeat arrhythmicity
Arrhythmia index (AI) = Standard deviation of HP / median HP
TRF delays age dependent deterioration of cardiac performance in *Drosophila*

Gill et al. Science 2015
Diet:
Protein 20%
Carb 40%
Fat 40%
Cholesterol 1.25%
TRF reduces atherosclerotic plaques in \( \text{LDLR}^{-/-} \) mice fed a high cholesterol diet.
Smartphone-app to monitor and intervene eating pattern
N=156 (65Males/91females), San Diego residents, No shiftworker, No students/employee or relatives of students/employees working at Salk. Average Age = 27.6y, Average BMI=24.74 Kg.Cm⁻²
Reminder push notifications 1/day:
“Did you eat or drink anything in the past 30min?”
“Yes/No”. Fraction of times when the subject answered “Yes” but there was no entry in 30min prior to the answer, is considered false negative rate; 10.34%.

Average eating or drinking lasts for ~14min. So all “events” recorded within 15min of another event is grouped into one “meal”.

Server side data analyses
Food annotation
Caloric vs non caloric intake
Portion size
Estimated calories
Taste modality (sweet, sour., umami etc.)
Place (home, work, restaurant)

Time-centric analyses
Total events: 26676
2.1% text entries, 97.9% pictures
22% (5846) water,
28% (7420) pre-packaged items with readily accessible nutrition information
50% (13410) were mixed meals with multiple items.
Average daily caloric intake
1947 Kcal; 95% CI: 1917-1977 (mean 1.233 fold over MC; 95% CI: 1.214-1.251)
(10.34% false reporting rate)

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects (n)</td>
<td>65</td>
<td>91</td>
<td>156</td>
</tr>
<tr>
<td>Age; years</td>
<td>26.4 (24.8-28.1)</td>
<td>28.4 (26.7-30.2)</td>
<td>27.6 (26.4-28.8)</td>
</tr>
<tr>
<td>Height; cm</td>
<td>177.1 (174.9-179.2)</td>
<td>163.5 (161.9-165)</td>
<td>169.1 (167.5-170.8)</td>
</tr>
<tr>
<td>Initial BMI</td>
<td>25.9 (24.69-27.11)</td>
<td>23.9 (22.86-24.95)</td>
<td>24.74 (23.94-25.53)</td>
</tr>
<tr>
<td>Final BMI (after 3 weeks)</td>
<td>25.82 (24.6-27.04)</td>
<td>23.87 (22.81-24.92)</td>
<td>24.68 (23.88-25.49)</td>
</tr>
<tr>
<td>Change in BMI (after 3 weeks)</td>
<td>-0.08</td>
<td>-0.04</td>
<td>-0.06</td>
</tr>
<tr>
<td>Paired t-test P-value</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>
Feedogram (raster plot of eating events)

Gill and Panda, Cell Metabolism 2015
Weekdays

6am  
Noon  
6pm  
Midnight

Weekends

Gill and Panda, Cell Metabolism 2015
Eating Duration

Gill and Panda, Cell Metabolism 2015
Weekend Metabolic Jetlag

Time of 1st caloric intake

- Mon-Fri
- Sat-Sun

Time of last caloric intake

- Mon-Fri
- Sat-Sun

40% delayed breakfast by >1 h,
25% delayed breakfast by > 2.1h
only 7% advanced breakfast by >1h

15% delayed last bite by >1 h
17% advanced last bite by >1h
% of Daily calories consumed by time X

<25% food is consumed between 4am-noon

>1/3rd calories consumed after 6pm

More (>25%) consumed between 6-9pm than between 4am-noon (<25%)
Time interval between waking up and first calorie

Time interval between last calorie and going to bed

% of All subjects

Cumulative percentage

Hours

0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5 10 10.5

0 10 20 30 40 50 60 70 80 90 100
Eating Pattern in urban India
Eating Pattern in urban India
Does BMI correlate with Eating Duration?

BMI does not show a simple correlation with eating duration

Many factors are known to contribute to a person’s BMI: Genetics, Epigenetics, Nutrient quantity, physical activity, etc.

We wanted to test in a subset of individuals that have BOTH >25 BMI and >14h eating duration, whether reduction in eating duration to 10 h without any overt suggestions on activity, or nutrition can reduce body weight.
Study Design

- **Baseline (3 Wks)**
  - 8 subjects with >14 h eating duration and BMI >25

- **Intervention (16 Wks)**

- **No monitoring (36 Wks)**

---

**Office visit:**
- Anthropometrics
- Questionnaire
Time Restricted Feeding (TRF) supports sustained weight loss and improvement in subjective quality of sleep.

Caloric containing eating duration (2.5-97.5%ile interval)

Body Weight (kg)

Subjective Score

Energetic - morning
Energetic - overall
Hunger at bedtime
Sleep satisfaction
Summary

A feasibility pilot study that shows individuals can restrict their eating duration to a self selected 10-11 h in natural living condition for several weeks.

TRF reduced body weight among a moderately overweight cohort by 3.8% that was sustained for a year.

However, Time restriction also led to reduced daily caloric intake by ~20%. So the study remains inconclusive whether TRF without reducing calories is beneficial. This may be tested in a controlled laboratory study.

If TR leads to CR, this method may be used to support caloric reduction among target at-risk population.
Erratic Lifestyle, Aging

Circadian lighting, Time Restricted Feeding
Welcome to myCircadianClock

A new type of app that helps you understand your body's rhythms while contributing to research.

Lifestyle is when, what, and how much you eat, sleep, and move.

Sign up for Free!  About this study  How this study works  What happens next
Open “myCircadianClock.org”

Tell us a little about yourself

If you consent to participate. You will get an activation code in your email

Download the “myCircadianClock” app from Appstore or Google play

Start using the app to log your food, sleep, and activity
<table>
<thead>
<tr>
<th>Circadian rhythm disruption or DIO</th>
<th>Time-restricted feeding</th>
<th>Potential mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obesity</td>
<td>↓Fat, ↑lean mass</td>
<td>↓Plasma- and ↓liver-triglycerides</td>
</tr>
<tr>
<td>Glucose intolerance/ insensitivity</td>
<td>Improved glucose homeostasis</td>
<td>↓Gluconeogenesis ↑PPP and ↑TCA cycle</td>
</tr>
<tr>
<td>Gut dysbiosis</td>
<td>Diverse and dynamic</td>
<td>Altered digestion, absorption, and excretion of nutrients and bile acids</td>
</tr>
<tr>
<td>Cardiovascular diseases</td>
<td>Arrhythmia and improved cardiac function*</td>
<td>ATP-dependent chaperone and improved mitochondria function</td>
</tr>
<tr>
<td>Chronic inflammation</td>
<td>↓Tissue inflammation</td>
<td>↓Macrophage infiltration of WAT ↓IL6 TNFα</td>
</tr>
<tr>
<td>Liver diseases</td>
<td>↓Fibrosis and ↓hepatic fat deposit</td>
<td>Fatty acid synthesis, ↑β oxidation mitochondrial volume</td>
</tr>
<tr>
<td>Increased cancer risk</td>
<td>↓Risk for breast cancer# and ↑breast cancer prognosis</td>
<td>Improved metabolic homeostasis, reduced inflammation</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>↓Cholesterol</td>
<td>Cholesterol metabolism to bile acids</td>
</tr>
<tr>
<td>Sleep disorders</td>
<td>↑Sleep quality* and ↑quantity*</td>
<td>Consolidation of activity and rest</td>
</tr>
<tr>
<td>Compromised muscle function</td>
<td>↑Endurance and ↑flight index*</td>
<td>Ketone bodies, creatine metabolism</td>
</tr>
</tbody>
</table>
Results: Cyclical Variation in the Gut Microbiome of Mice on a Normal Chow Ad Libitum Diet

Results: Cyclical Variation in the Gut Microbiome of Mice on a Normal Chow Ad Libitum Diet

Results: Cyclical Variation is Dampened in Mice on a High Fat Ad Libitum Diet

Results: Cyclical Variation is Dampened in Mice on a High Fat Ad Libitum Diet

Results: Cyclical Variation at Phylum Level is Not Restored in Time Restricted Feeding Mice

Results: Cyclical Variation at Phylum Level is Not Restored in Time Restricted Feeding Mice

Results: Daily Shifts in NA Gut Microbiome
Results: Daily Shifts in NA Gut Microbiome

Feeding: Erysipelotrichaceae

Fasting: Akkermansia

FA or FT: Lachnospiraceae

Results: TRF Changes Reads in Gut Microflora Associated with Dysmetabolism

Results: TRF Changes Reads in Gut Microflora Associated with Dysmetabolism

Reduced in FT: lactobacillus

Increased in FT: Ruminococcaceae

Results: Gut Microbiome Provides Enzymes Necessary for Digestion of Complex Sugars

Complex Sugars

- Xylose
- Galactose

Bacterial Enzymes
Results: But Xylose and Galactose are Excreted in Stool with TRF
Results: FT Mice Excrete More Bile Acids

Bile acids can affect lipid, cholesterol, and glucose metabolism via intestinal and hepatic farsenoid X receptor.

Why isn’t there more cycling in the time restricted feeding condition?
Functional Analysis of Cecal Content: Metatranscriptome

How many unique “species” are there?

Zarrinpar, et al. Unpublished
Functional Analysis of Cecal Content: Metatranscriptome

Compositional analysis:
Who’s there?
What genes are available?

Functional analysis:
What genes are being expressed?
What are they doing?

- HUMAnN2 – new bioinformatic tools to identify function of bacterial gene transcripts from stool

50 million PE reads -> 570,000 transcripts -> 2081 pathways

Zarrinpar, et al. Unpublished
Cyclical Fluctuation of Transcriptome

Total Pathways Represented (% from 2081)

Compositional Analysis (DNA)
OTUs
- NA: 83% (17% Cyclical, 83% Non-Cyclical)
- FA: 93% (7% Cyclical, 93% Non-Cyclical)
- FT: 91% (9% Cyclical, 91% Non-Cyclical)

Functional Analysis (RNA) Pathways
- 98% Non-Cyclical

Zarrinpar, et al. Unpublished
Cyclical Fluctuation of Transcriptome

Total Pathways Represented (% from 2081)

Compositional Analysis (DNA) OTUs

- NA: 83% Non-Cyclical, 17% Cyclical
- FA: 93% Non-Cyclical, 7% Cyclical
- FT: 91% Non-Cyclical, 9% Cyclical

Functional Analysis (RNA) Pathways

- NA: 98% Non-Cyclical, 2% Cyclical
- FA: 100% Non-Cyclical
- FT: 98% Non-Cyclical, 2% Cyclical

Zarrinpar, et al. Unpublished
Cyclical Fluctuation of Transcriptome

Compositional Analysis (DNA) OTUs
- NA: 83% Non-Cyclical, 17% Cyclical
- FA: 93% Non-Cyclical, 7% Cyclical
- FT: 91% Non-Cyclical, 9% Cyclical

Functional Analysis (RNA) Pathways
- NA: 98% Non-Cyclical, 2% Cyclical
- FA: 100% Non-Cyclical
- FT: 87% Non-Cyclical, 13% Cyclical

Total Pathways Represented (% from 2081)
- NA: 79%
- FA: 37%
- FT: 67%

Zarrinpar, et al. Unpublished
Feeding Pattern Affects Gut Metatranscriptome

Zarrinpar, et al. Unpublished
Feeding Pattern Affects Gut Metatranscriptome

Zarrinpar, et al. Unpublished
Feeding Pattern Affects Gut Metatranscriptome

Zarrinpar, et al. Unpublished
Follow up: Transcripts of Interest

Bile Acid degradation

Cellulose degradation

Zarrinpar, et al. Unpublished
Potential Mechanism: Time Restricted Feeding and Bile Acid Signaling

FXR also affects lipid, triglyceride, and glucose metabolism.

Potential Mechanism: Time Restricted Feeding and Bile Acid Signaling

Gilardi, Pharm & Therap 2007; Matsubara, Mol Cell End, 2012;
Calkin, Nat Rev Mol Cell Bio, 2012
Hypotheses

Antibiotic induced microbiome depletion (AIMD) alters luminal short chain fatty acids (SCFAs) and bile acids (BAs).

AIMD alters gut luminal signaling.

AMID affects host glucose homeostasis
Summary Part 2: Cyclical Dynamics of the Gut Microbiome.

The Relationship Between Gut Microbiome and Gut Signaling in Metabolism

Gut microflora

Diet/Feeding Pattern

Gut metabolome

Direct Effect

Indirect effect

Gut signaling (e.g. TLR, Bile acid)

Liver

Skeletal Muscle

Brain

Adipose Tissue

Bile acids
Saturated Fatty acids
Short chain fatty acids