Circadian Clocks, Metabolism and Disease
Hee-Kyung Hong
Joe Bass Laboratory (Northwestern University)
The Use of Biology and Energy Drinks Workshop
August 15, 2013
Caffeine and increased caloric intake alters circadian clocks.

- Chronic caffeine consumption lengthens the period of circadian locomotor rhythms in mice and alters clock gene expression.
  
  Oike et al., *Biochem Biophys Res Commun*. 2011

- Diet-induced obesity is a key risk factor for a variety of chronic conditions, including diabetes, hypertension, high cholesterol, stroke, cardiovascular disease and cancer.
  
  Alberti et al., *Circulation*, 2009

- Poor-quality sleep is associated with elevated BMI and development of metabolic disorders.
  
  Van Cauter et al., *Eur J Endocrinol*, 2008

America’s top 7 favorite energy drinks

<table>
<thead>
<tr>
<th>PRODUCT (8 fluid oz)</th>
<th>Calories</th>
<th>Sugars (g)</th>
<th>Caffeine (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Red Bull Energy Drink</td>
<td>105</td>
<td>26</td>
<td>79</td>
</tr>
<tr>
<td>2. Monster Energy</td>
<td>100</td>
<td>27</td>
<td>92</td>
</tr>
<tr>
<td>3. Rockstar Energy Drink Double</td>
<td>140</td>
<td>31</td>
<td>80</td>
</tr>
<tr>
<td>4. NOS High Performance Energy Drink</td>
<td>110</td>
<td>26</td>
<td>112</td>
</tr>
<tr>
<td>5. Amp Energy</td>
<td>110</td>
<td>29</td>
<td>71</td>
</tr>
<tr>
<td>6. Full Throttle</td>
<td>220</td>
<td>58</td>
<td>210</td>
</tr>
<tr>
<td>7. Xyience Xenergy</td>
<td>0</td>
<td>0</td>
<td>94</td>
</tr>
</tbody>
</table>
I. Overview of circadian rhythms and core clock machinery

II. Integration of circadian rhythms and metabolism

III. Circadian disruption and disease

IV. Role of the circadian system in metabolism
   a. Glucose metabolism and insulin secretion
   b. Molecular control of Sirt1 and NAD: impact on mitochondrial oxidative metabolism
   c. Impact of high fat diet on circadian rhythm: differential effects of saturated and unsaturated fatty acids
I. Circadian Rhythms

- Derived from Latin ‘circa diem’ (about a day)
- Defined as a biological rhythm that persists under constant conditions with a period length of ~24 hrs
- Mammalian circadian clock orchestrates the synchronization of the daily behavioral and physiological rhythms to better adapt the organism to the external environment
Genetic Basis of Timing

Clock Mutation Results in Loss of Behavioral Rhythms

- Clock mouse was identified in an ENU mutagenesis screen
- Light/Dark cues are sufficient to maintain activity rhythms in Clock mice
- Free running behavior rhythms are lost

Vitaterna et al., Science, 1994
King et al., Cell, 1997
Mammalian circadian clock is composed of a cell autonomous transcriptional-translational feedback network
Mammalian Circadian Pacemaker

Light

Suprachiasmatic Nucleus (SCN)

Output Rhythms:
Physiology
Behavior

National Institute of General Medical Sciences
Yoo et al., (2004) PNAS

- Sleep/Activity
- Thermoregulation
- Food consumption
- Blood pressure
- Hormonal release
- Metabolism

Central vs. Peripheral (local) Oscillators

Per2::Luciferase Rhythm
Circadian Rhythms and Glucose Metabolism in Humans

- Blood glucose levels peak at the onset of the active period
  
  *Arslanian et al, Horm Res, 1990; Bolli et al, Diabetes, 1984*

- Glucose tolerance is impaired in evening compared to morning hours
  
  *Gagliardino et al, Chronobiologia, 1984*

- Decreased insulin secretion and altered insulin sensitivity in the evening
  
  *Boden et al, Am J Physiol, 1996*

- Daily cycles of insulin secretion and sensitivity are lost in diabetic patients
  
  *Boden et al, Diabetes, 1999*
II. *Clock* Mutants Provide Genetic Evidence Linking Circadian Rhythmicity and Metabolism

- Hyperdipidemia
- Susceptible to diet-induced obesity
- Age-dependent hyperglycemia
- Hypoinsulinemia at young age

## III. Circadian Mutations and Metabolic Disease (mouse studies)

<table>
<thead>
<tr>
<th>Whole Body Mutation</th>
<th>Circadian Phenotype</th>
<th>Cardiometabolic Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clock</em> Δ19/Δ19</td>
<td>Arrhythmic</td>
<td>Age-dependent hyperphagia, obesity, hyperlipidemia, hyperglycemia, hypoinsulinemia, reduced muscle strength, endothelial dysfunction</td>
<td>PMID 11163178, 8171325, 20956306, 20562852, 15845877, 19273720</td>
</tr>
<tr>
<td><em>Bmal1</em> −/−</td>
<td>Arrhythmic</td>
<td>Loss of glucose and triglyceride oscillations, increased insulin sensitivity, hypoinsulinemia, increased vascular stiffness, thrombosis, endothelial dysfunction, age-associated dilated cardiomyopathy, reduced muscle strength</td>
<td>PMID 22707558, 20829506, 20956306, 20562852, 15523558, 19273720</td>
</tr>
<tr>
<td><em>Per1</em> −/−</td>
<td>Arrhythmic</td>
<td>Impaired glucocorticoid rhythm</td>
<td>PMID 11389837, 16505983</td>
</tr>
<tr>
<td><em>Per2</em> −/−</td>
<td>Arrhythmic</td>
<td>Aortic endothelial dysfunction</td>
<td>PMID 11389837, 17404161</td>
</tr>
<tr>
<td><em>Per1/Per2/Per3</em> −/−</td>
<td>Arrhythmic</td>
<td>Increased vascular stiffness</td>
<td>PMID 20829506</td>
</tr>
<tr>
<td><em>Cry1/Cry2</em> −/−</td>
<td>Arrhythmic</td>
<td>Salt-sensitive hypertension</td>
<td>PMID 10217146, 20023637</td>
</tr>
<tr>
<td><em>Per2/Cry1</em> −/−</td>
<td>Arrhythmic</td>
<td>Loss of ACTH and glucocorticoid rhythm</td>
<td>PMID 16890544</td>
</tr>
<tr>
<td><em>Rev-erbα</em> −/−</td>
<td>Arrhythmic</td>
<td>Impaired bile acid synthesis, hepatic steatosis</td>
<td>PMID 19721697, 21393543</td>
</tr>
<tr>
<td><em>Rev-erbα/Rev-erbβ</em> −/−</td>
<td>Arrhythmic</td>
<td>Increased glucose and triglycerides, reduced circulating fatty acids, reduced respiratory exchange ratio</td>
<td>PMID 22460952</td>
</tr>
</tbody>
</table>
Genetic Basis of Timing

- Mouse Clock Mutant
  King et al., Cell, 1997

- Human *Per2* was discovered in people with Familial Advance Sleep Phase Syndrome
  Toh et al., Science, 2001
# Circadian Gene Polymorphisms and Metabolic Phenotypes

(Human Genome-Wide Association Studies)

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Metabolic Association</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CLOCK</strong></td>
<td>Hypertension, obesity, metabolic syndrome, non-alcoholic fatty liver disease, high plasma ghrelin, short sleep duration, altered eating behaviors, higher total energy intake, decreased compliance with prescribed diet plans, resistance to weight loss</td>
<td>PMID: 17696255, 18541547, 20653450, 20497782, 9846548, 19888304</td>
</tr>
<tr>
<td><strong>BMAL1</strong></td>
<td>Hypertension and type 2 diabetes</td>
<td>PMID: 17728404</td>
</tr>
<tr>
<td><strong>CRY2</strong></td>
<td>Altered fasting glucose concentrations</td>
<td>PMID: 20081858</td>
</tr>
<tr>
<td><strong>PER2</strong></td>
<td>Hyperglycemia, abdominal obesity, unhealthy feeding behavior, waist circumference and cholesterol levels</td>
<td>PMID: 19470168, 20205566, 20497782, 17653067</td>
</tr>
<tr>
<td><strong>NAMPT1</strong></td>
<td>Protection from obesity</td>
<td>PMID: 19300429</td>
</tr>
<tr>
<td><strong>PER2/PER3/CLOCK/BMAL1</strong></td>
<td>Morning or evening activity preference</td>
<td>PMID: 21637568</td>
</tr>
<tr>
<td><strong>MTNR1A or MTNR1B</strong></td>
<td>High fasting glucose levels, impaired insulin secretion, increased risk of type 2 diabetes, insulin resistance and susceptibility to polycystic ovarian syndrome</td>
<td>PMID: 12957828, 21474908, 1470412, 21112029, 19241057, 19937311, 20628598, 21658282, 22233651, 21474908, 19060908, 19088850</td>
</tr>
</tbody>
</table>
IV. Circadian Regulation of Metabolism

Zeitgebers
- Light
- Food

Clock Oscillator
- Brain
- SCN
- extra-SCN
- Endocrine or Autonomic Innervation

Clock Output
- Feeding
- Sleep-Wake Cycle
- Hormones
- Metabolic Pathways

Adapted from Ramsey et al, Annu Rev Nutr, 2007
I. Overview of circadian rhythms and core clock machinery

II. Integration of circadian rhythms and metabolism

III. Circadian disruption and disease

IV. Role of the circadian system in metabolism
   a. Glucose metabolism and insulin secretion
   b. Molecular control of Sirt1 and NAD: impact on mitochondrial oxidative metabolism
   c. Impact of high fat diet on circadian rhythm: differential effects of saturated and unsaturated fatty acids
IV a. Glucose metabolism and insulin secretion: Identification of Circadian Gene Oscillation in Pancreatic Islets

- Circadian clock is expressed autonomously within the pancreatic islet
Development of Age-Dependent Diabetes in *Clock* Mutant Mice

Marcheva et al., *Nature*, 2010

- Impaired glucose tolerance
- Reduced islet size, proliferation, and insulin release

**a** Glucose tolerance test: glucose levels (ZT14)

**b** Glucose tolerance test: insulin levels (ZT14)

**c** Islet histology

**d** Islet size
Multiple circadian genes impact the capacity of the pancreatic islets to respond to glucose

Marcheva et al., Nature, 2010

**a** Pancreas-specific *Bmal1* knockout: islet staining

*PdxCre*  
*PdxCre Bmal^flx/flx*

**b** Glucose tolerance test: glucose levels (ZT2)

**c** Glucose tolerance test: insulin levels (ZT2)
Potential Sites for Clock Gene Action in Glucose Stimulated Insulin Secretion

1. Beta-Cell Development
   - Mutant CLOCK
   - BMAL1
   - CACGTG

2. Insulin Production
   - Beta-cell
   - INSULIN

3. Insulin Secretion
   - Beta-cell
IV b. Molecular Clock Control of SIRT1 and NAD

Ramsy et al., Science, 2009

[Diagram of molecular clock control of SIRT1 and NAD]

Circadian Oscillation of Nampt & NAD in Constant Darkness in WT and Clock mutant Mice

Ramsy et al., Science, 2009
SIRT3 regulates mitochondrial oxidative function
Does the circadian clock regulate mitochondrial oxidative metabolism through rhythms of NAD+-driven SIRT3 activity?
High-Fat Diet Disrupts Behavioral and Molecular Circadian Rhythms in Mice

Akira Kohsaka,1,4 Aaron D. Laposky,1,2 Kathryn Moynihan Ramsey,1,3,4 Carmela Estrada,1 Corinne Joshu,1 Yumiko Kobayashi,4 Fred W. Turek,1,2 and Joseph Bass1,2,3,4,*

Cell Metabolism

(2007)
IV c. High-fat diet affects behavioral rhythms and attenuates clock gene expressions in metabolic tissues

Mammalian circadian clock

Adapted from Marcheva et al, J Appl Phys, 2009

Kohsaka et al. (2007)
Question: Are these disruptions of behavior due to the fat content of the diet?

Cell Metabolism
Correspondence

Comparisons of Diets Used in Animal Models of High-Fat Feeding

Craig H. Warden, and Janis S. Fisler
Cell Metabolism (2008)

“Many papers using animal models draw conclusions about dietary effects from comparison of natural-ingredient chow with defined diets, despite marked difference in micro and macronutrient content.”
Does fatty acid composition of diet have differential effects on circadian period? UFD vs. SFD

Maury et al, SRBR meeting (2012)

Kcal (%)

<table>
<thead>
<tr>
<th></th>
<th>RC</th>
<th>UFD</th>
<th>SFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>27</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>57</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Fat</td>
<td>16</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Saturated fatty acids (C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C20)</td>
<td>6.4</td>
<td>26.3</td>
<td></td>
</tr>
<tr>
<td>Monounsaturated fatty acids (C14:1, C16:1, C18:1, C20:1)</td>
<td>29.8</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td>Polyunsaturated fatty acids (C18:2, C18:3)</td>
<td>8.8</td>
<td>5.2</td>
<td></td>
</tr>
</tbody>
</table>

B

* $p = 0.025$, paired
** $p = 0.015$

n=20 / group
Differential effects of UFD and SFD on daytime activity

Maury et al, SRBR meeting (2012)
Fatty acid composition in diet affects properties of cell autonomous circadian oscillator

Per2::Luc genetic reporter mouse fed on UFD or SFD

4 weeks on diet

SCN dissection

Or peripheral tissues

Ex vivo culture

luminometry

Relative count

days

Yoo et al. (2004)

Diets enriched in saturated fatty acids

lengthens

behavioral rhythm and molecular clock in SCN.

A

B

Maury et al, SRBR meeting (2012)
Acknowledgement

Environment factors contributing to circadian misalignment

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