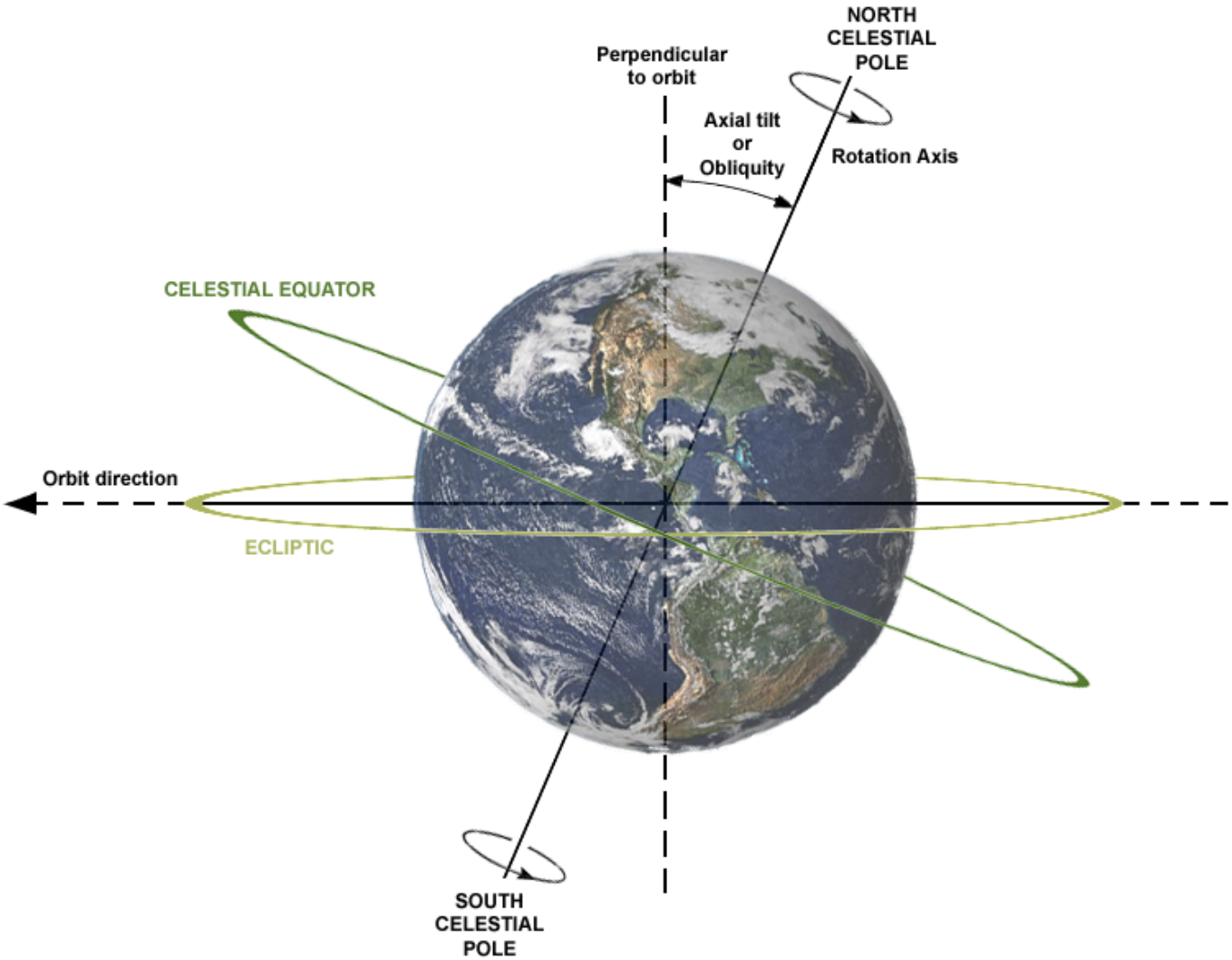
Metabolomics in chronobiology: metabolic alterations by sleep loss and circadian function

Aalim M. Weljie

aalim@pennmedicine.upenn.edu

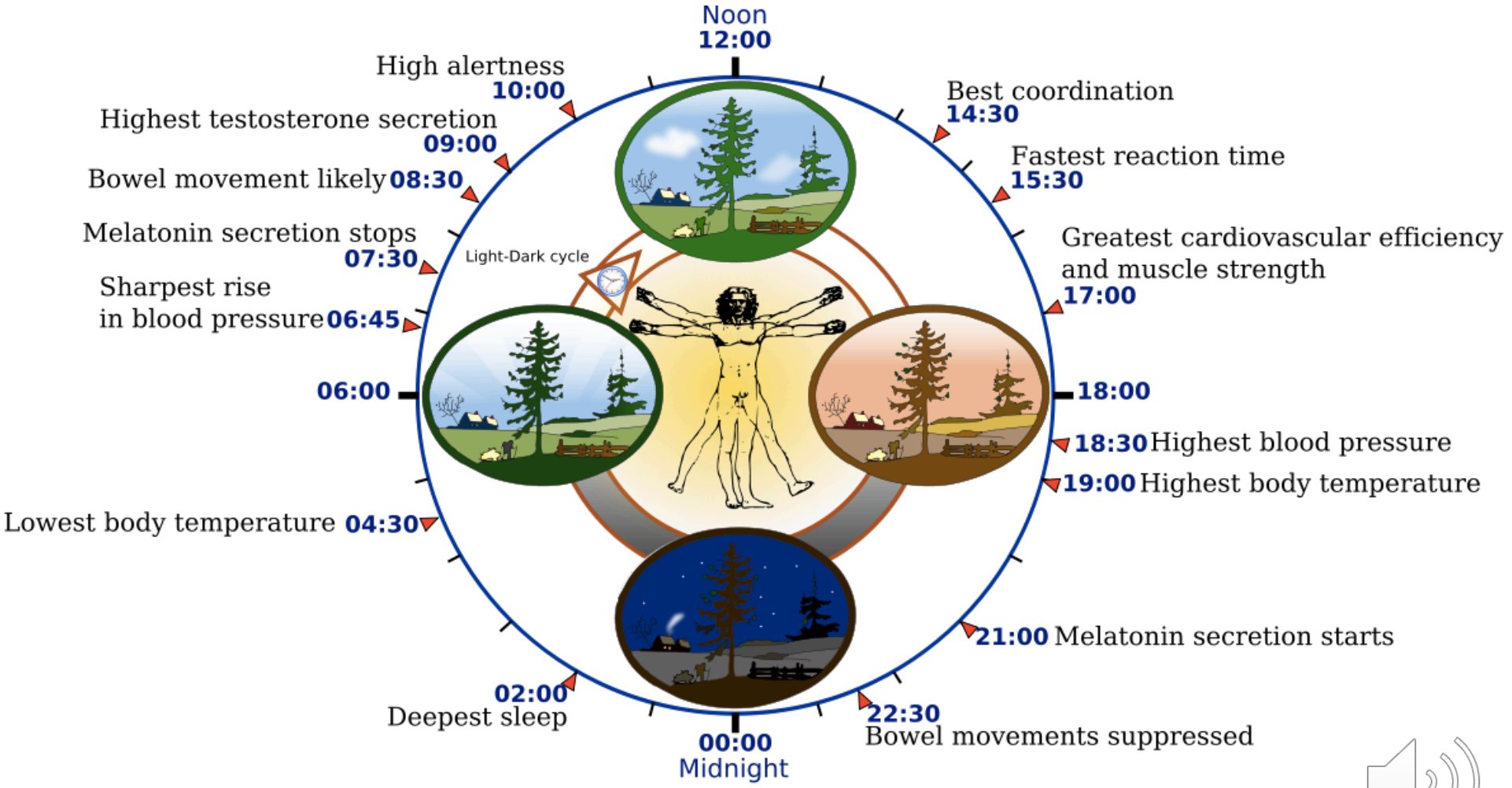
Department of Systems Pharmacology and Translational Therapeutics

Life has evolved in the context of constant, rhythmic oscillations



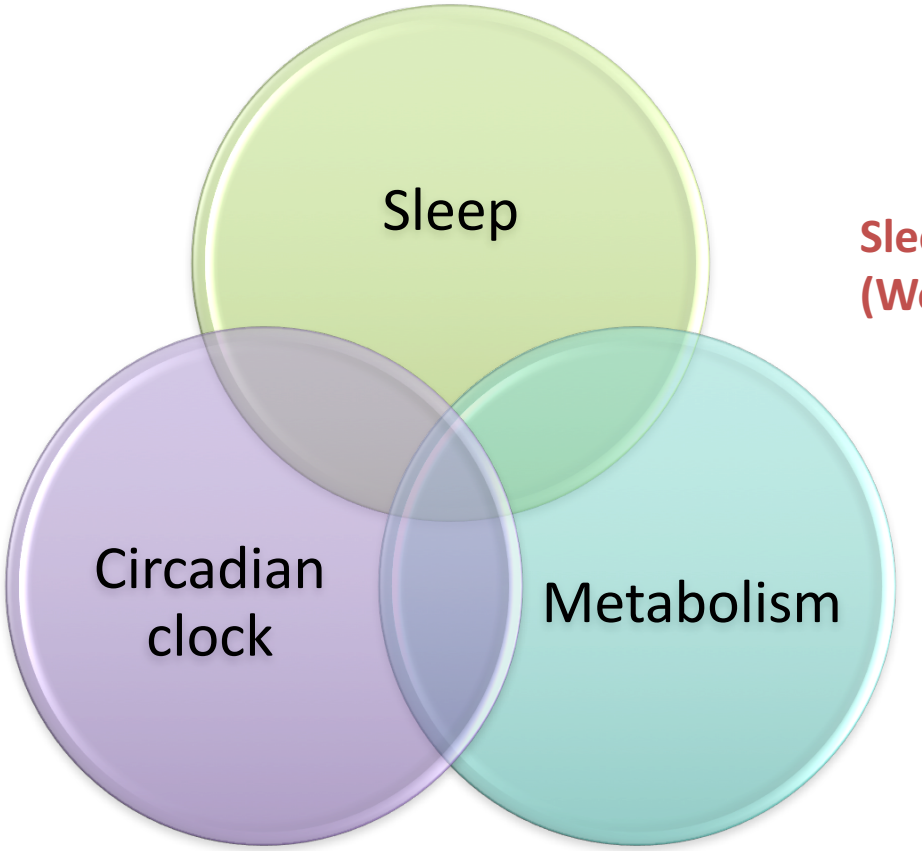
Dennis Nilsson, wikimedia

Our behaviors are coordinated in a rhythmic fashion



wikimedia

Triad of sleep / circadian rhythms and metabolism increasingly implicated in disease processes



Sleep Deprivation (Weljie et al, PNAS, 2015)

We live in an increasingly sleep restricted and disrupted world

- stress
- high work load
- shift work
- home work
- parenthood
- recreation

• Immediate effects on alertness, cognitive function and emotional regulation

• Gradually developing maladaptive changes in brain and body that may increase the sensitivity to disease

Peter Meerlo

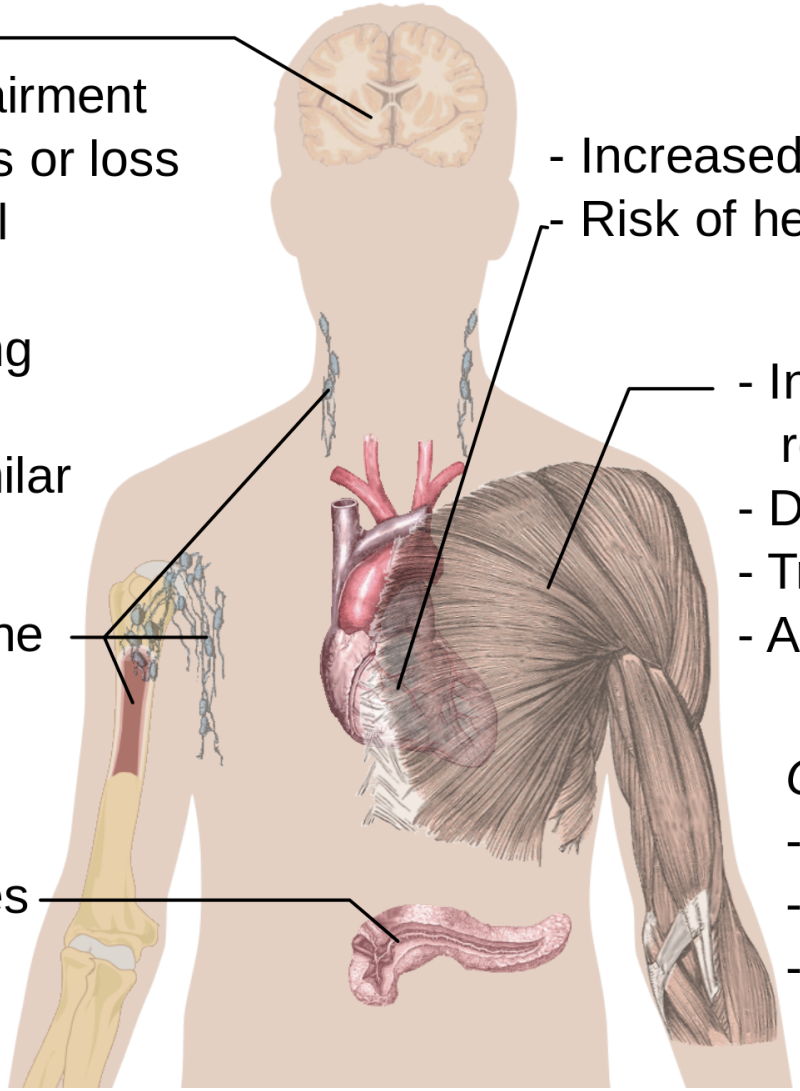
Effects of

Sleep deprivation

- Irritability
- Cognitive impairment
- Memory lapses or loss
- Impaired moral judgement
- Severe yawning
- Hallucinations
- Symptoms similar to ADHD

- Impaired immune system

- Risk of diabetes Type 2



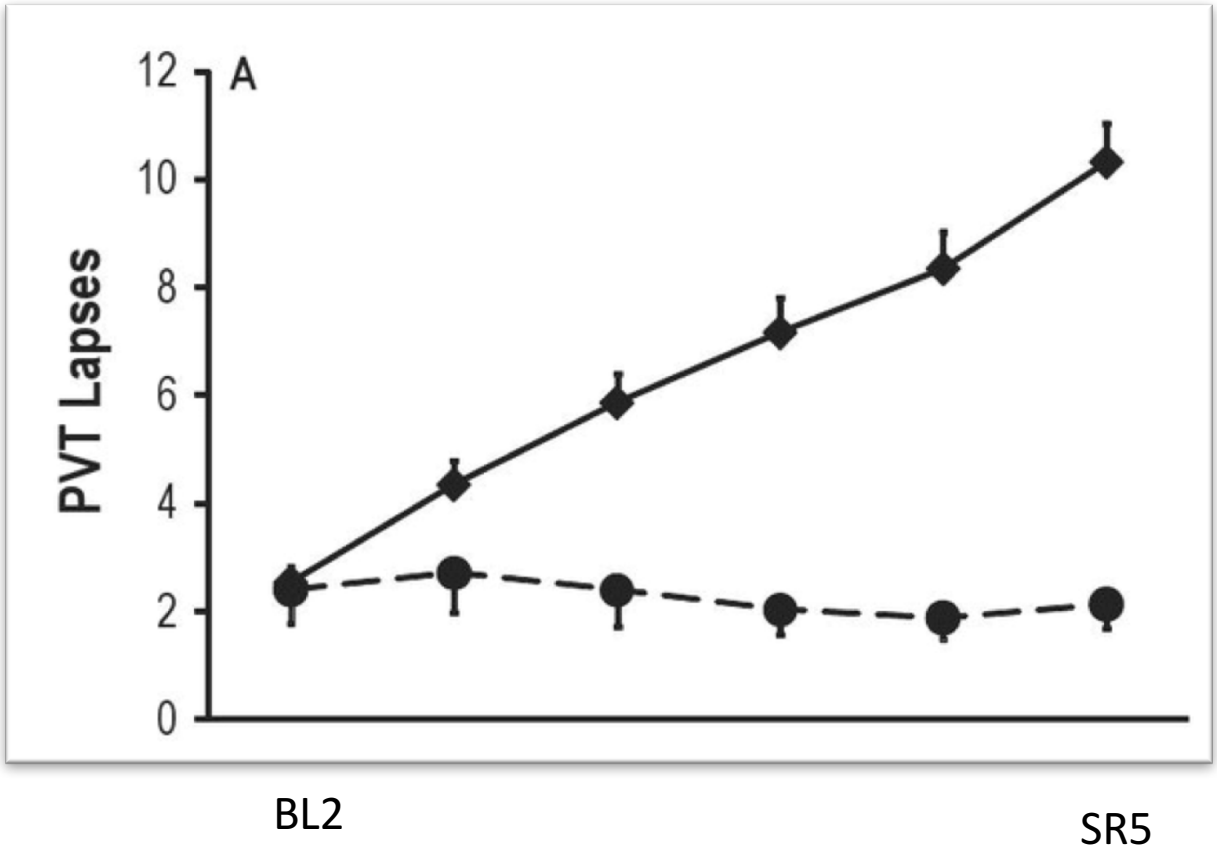
- Increased heart rate variability
- Risk of heart disease

- Increased reaction time
- Decreased accuracy
- Tremors
- Aches

Other:
- Growth suppression
- Risk of obesity
- Decreased temperature

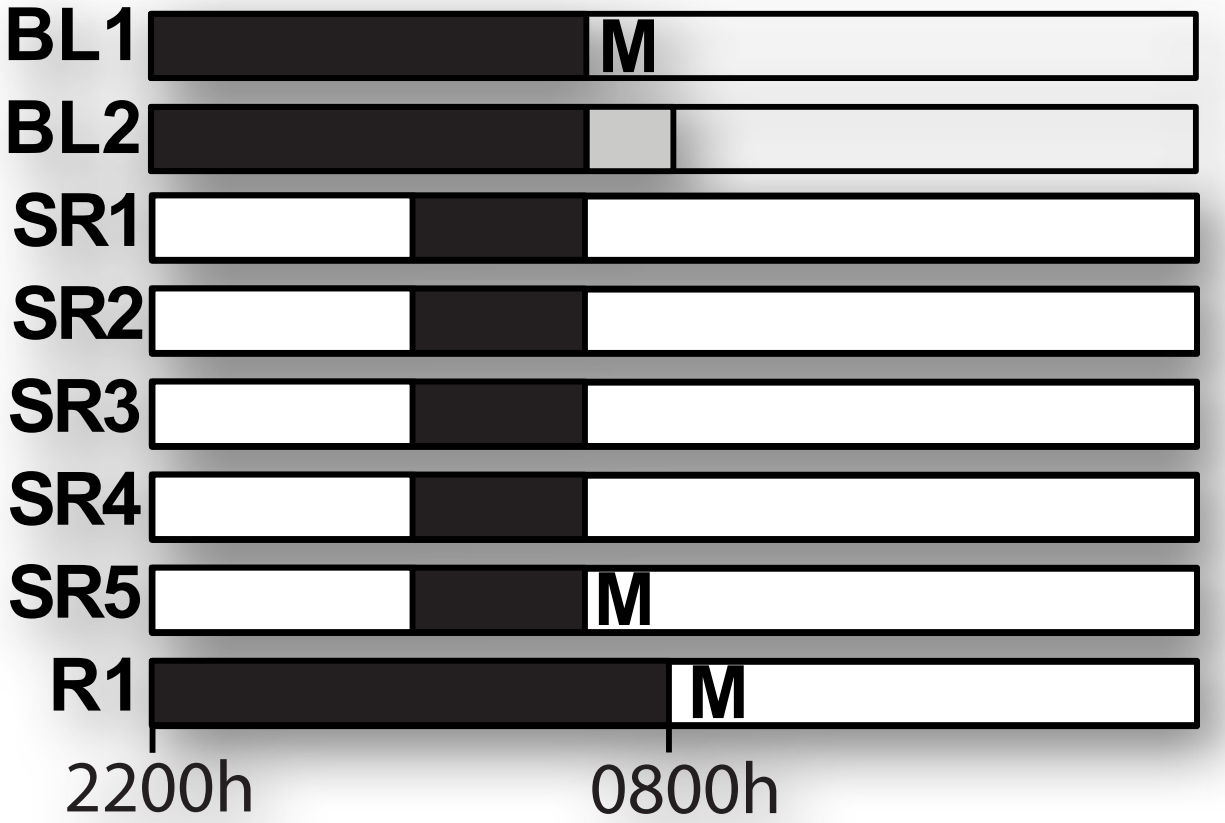
wikimedia

Psychomotor vigilance task quantitatively demonstrates cognitive changes with sleep deprivation



Banks S; Van Dongen HPA; Maislin G; Dinges DF. Neurobehavioral dynamics following chronic sleep restriction: dose-response effects of one night for recovery. SLEEP 2010;33(8):1013–1026.

Serum sampled from human subjects with a baseline sample, 5 nights of SR and one recovery sleep opportunity



David Dinges, Namni Goel, UPenn

Weljie, et al. *PNAS* 2015

Comprehensive analytical analysis

GCMS

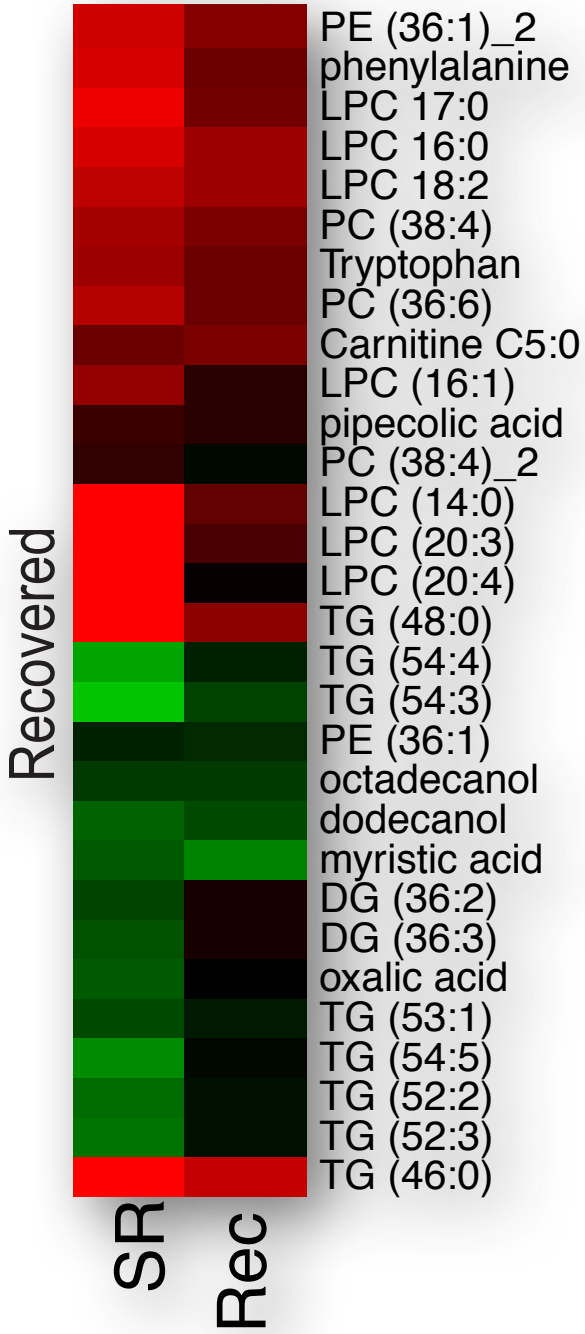
- Fatty acids
- Organic acids
- Amino acids

HILIC LCMS

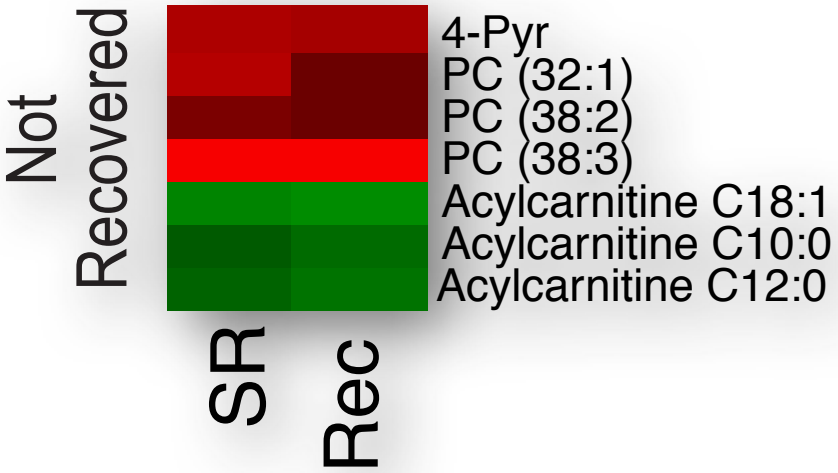
- Charged moieties
- Polar molecules
- Short / medium carnitines, LPCs, LPEs, and PCs

RP-LCMS

- Non-polar
- Lipids
- Fatty acids



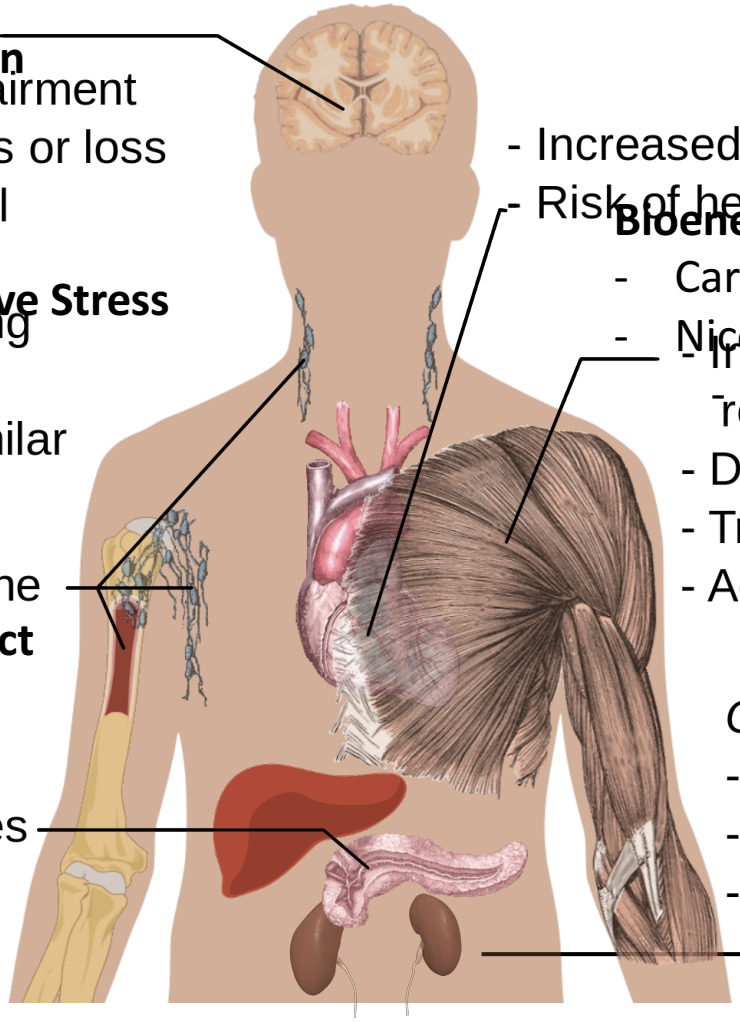
Most metabolites altered under restricted sleep recover– but not all



Weljie, et al. *PNAS* 2015

Metabolite-centric view of sleep deprivation effects

Effects of
Sleep deprivation



Neurotransmission
- Phe, Trp

- Irritability
- Cognitive impairment
- Memory lapses or loss
- Impaired moral judgement

Systemic Oxidative Stress
- Severe yawning
- Hallucinations
- Symptoms similar to ADHD

- Impaired immune system

Gastrointestinal impact
- Gut microflora
  - Pipecolic acid
- Lipid metabolism
  - Risk of diabetes Type 2
  - Lipids,
  - Fatty acids and
  - alcohols

- Increased heart rate variability
- Risk of heart disease

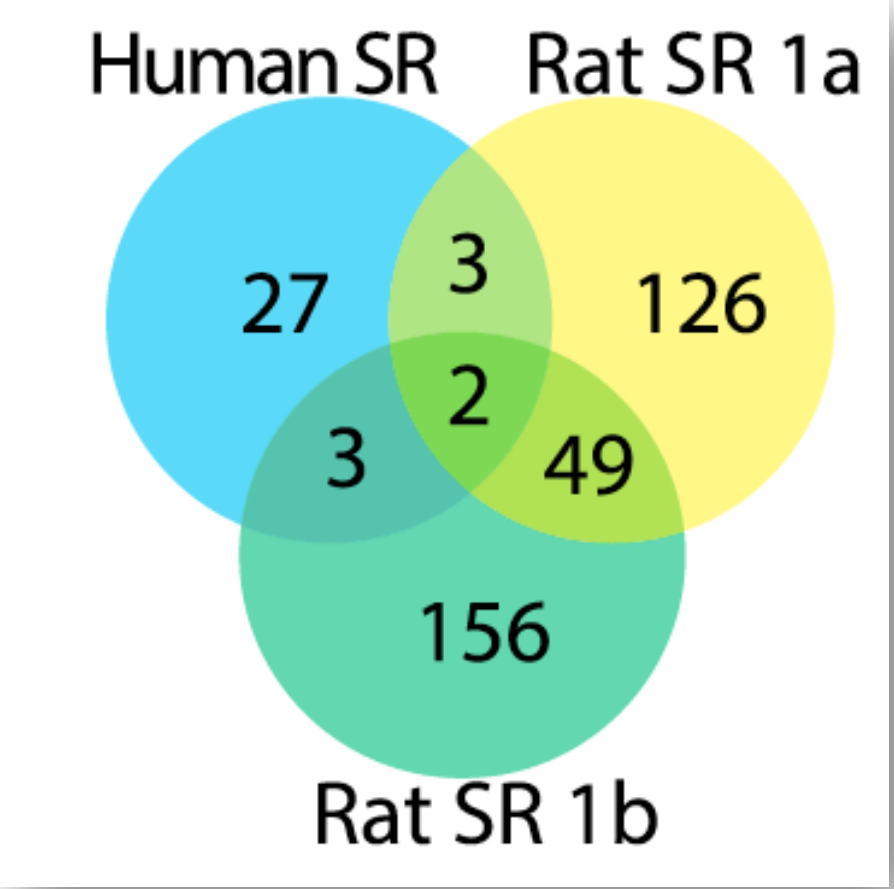
Bioenergetics
- Carnitines, Lipids
- Nicotinamide metabolism
  - Trp, 4-pyr
- Increased reaction time
- Decreased accuracy
- Tremors
- Aches

Other:
- Growth suppression
- Risk of obesity
- Decreased temperature

Renal metabolism
- Oxalate

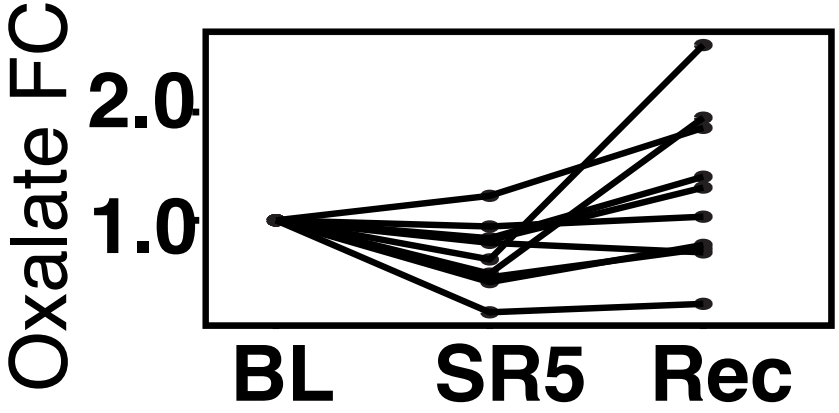
Wikimedia

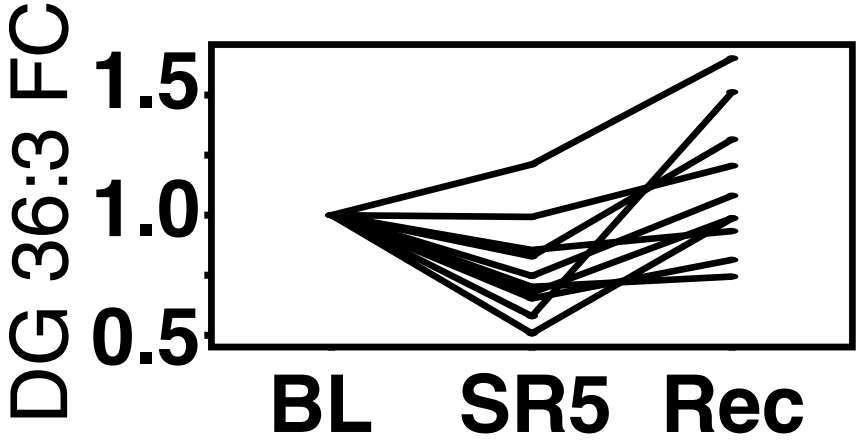
Two metabolites altered in both rats and humans…



Weljie, et al. *PNAS* 2015

Oxalic acid and diacylglycerol 36:3

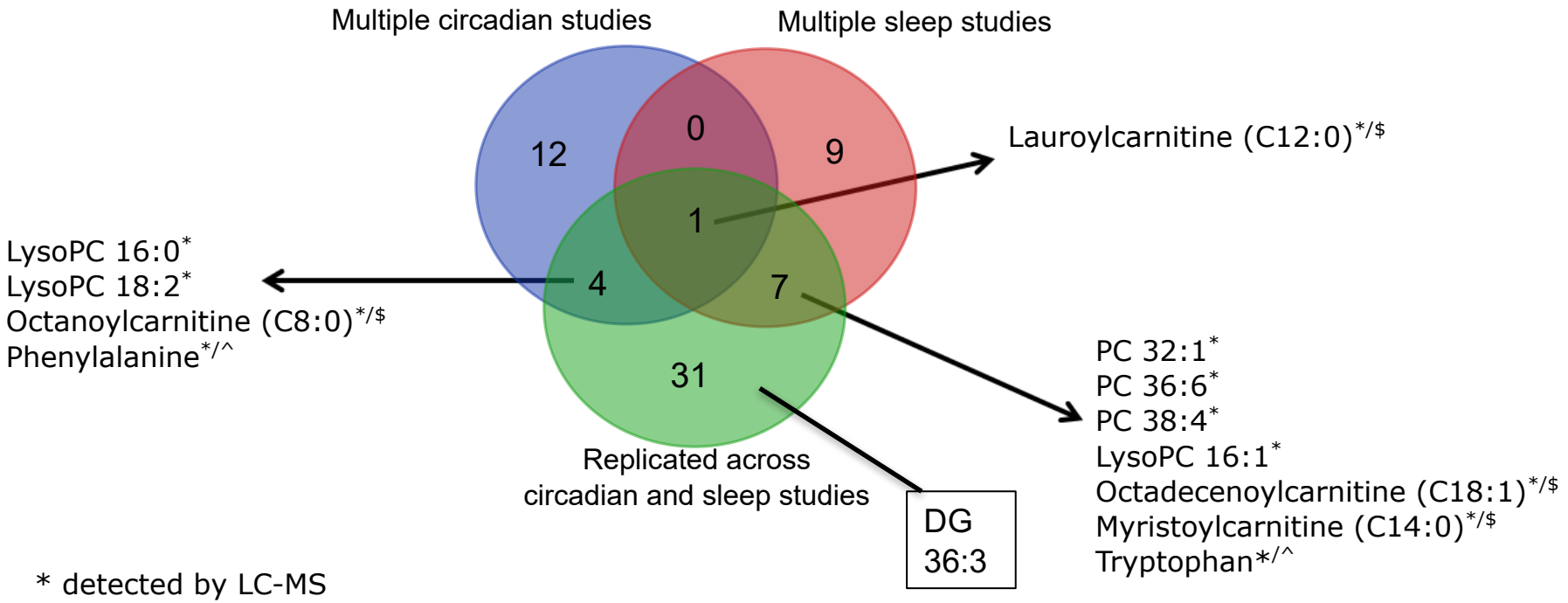




Metabolite	Rat			Human		
	P-value	FDR	Day	P-value	FDR	Day
Oxalic acid	<0.01	0.02	1↓	<0.01	0.19	5↓
DG 36:3	<0.01	0.008	1↓	<0.01	0.12	5↓

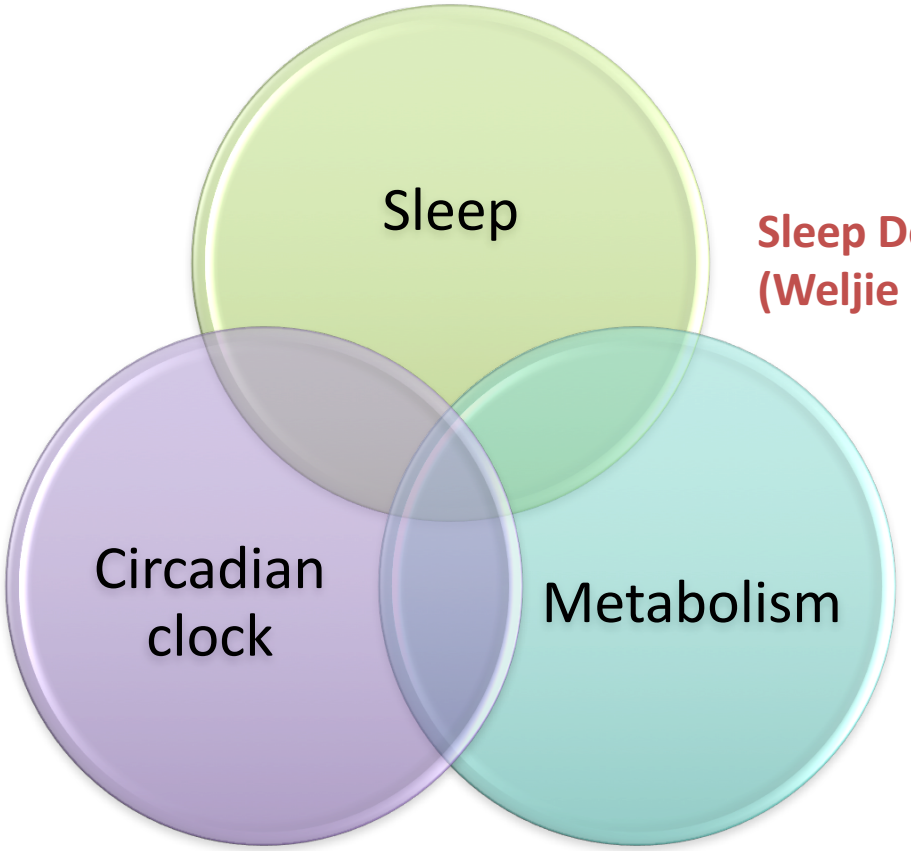
Weljie, et al. *PNAS* 2015

Analysis of human sleep and circadian studies show significant overlaps– but also significant opportunity to move beyond the low hanging fruit



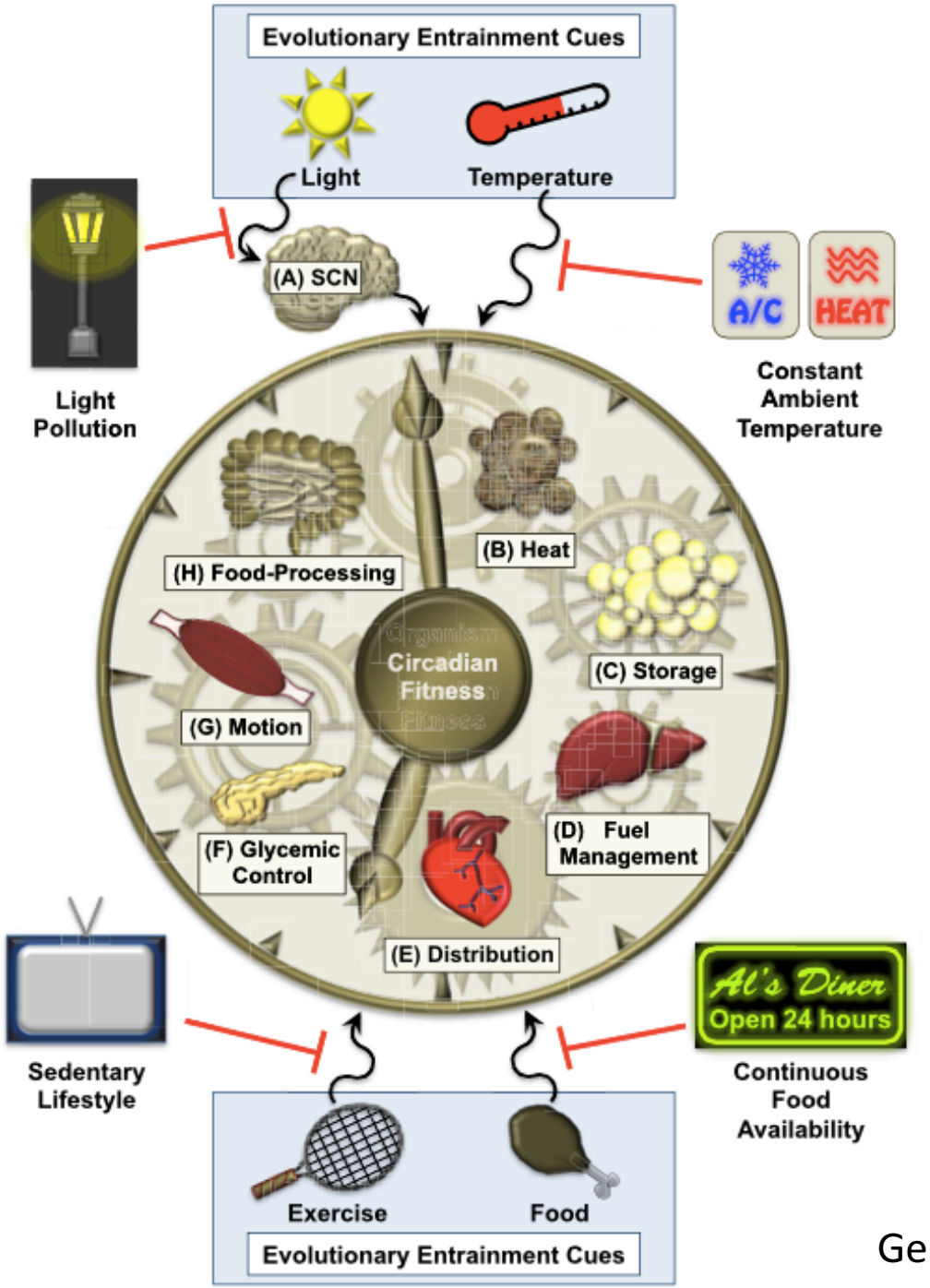
Rhoades, Sengupta, Weljie, Current Opinion in Biotech, 2017

Triad of sleep / circadian rhythms and metabolism increasingly implicated in disease processes



Sleep Deprivation
(Weljie et al, PNAS, 2015)

Clock-driven metabolism
(Krishnaiah et al., Cell Metabolism 2017)
(Skarke et al., Submitted)



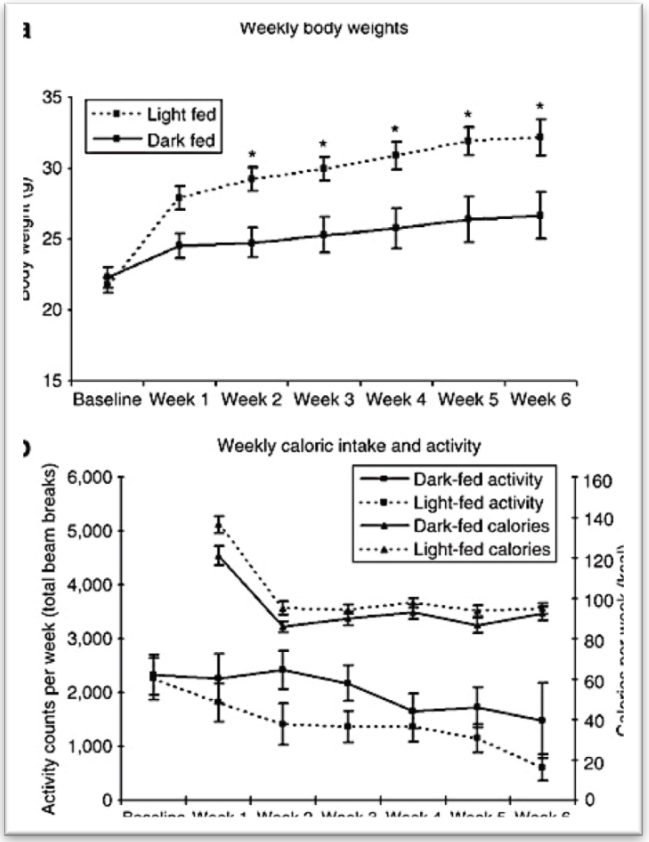
The circadian system works bi-directionally with metabolism to maintain energetic homeostasis

Disruption is implicated in numerous disease states

Gerhart-Hines, Lazar *Endocrine Reviews*, 2015

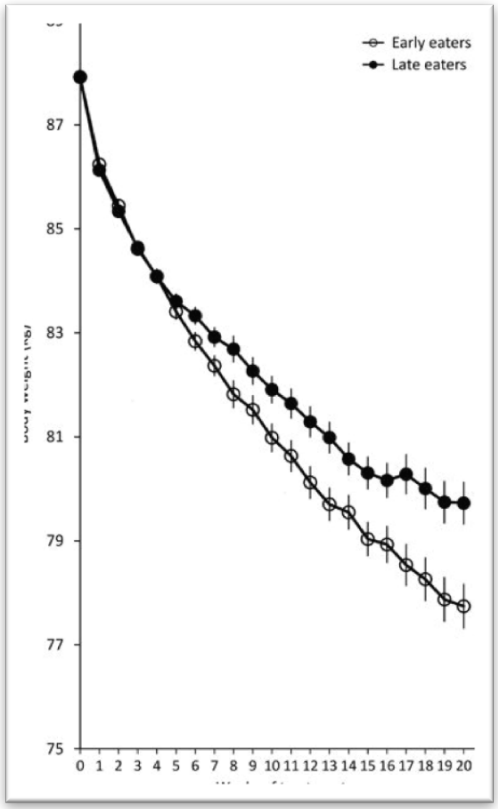
Examples: timing of food intake changes overall energy balance

Mis-timed feeding in mice



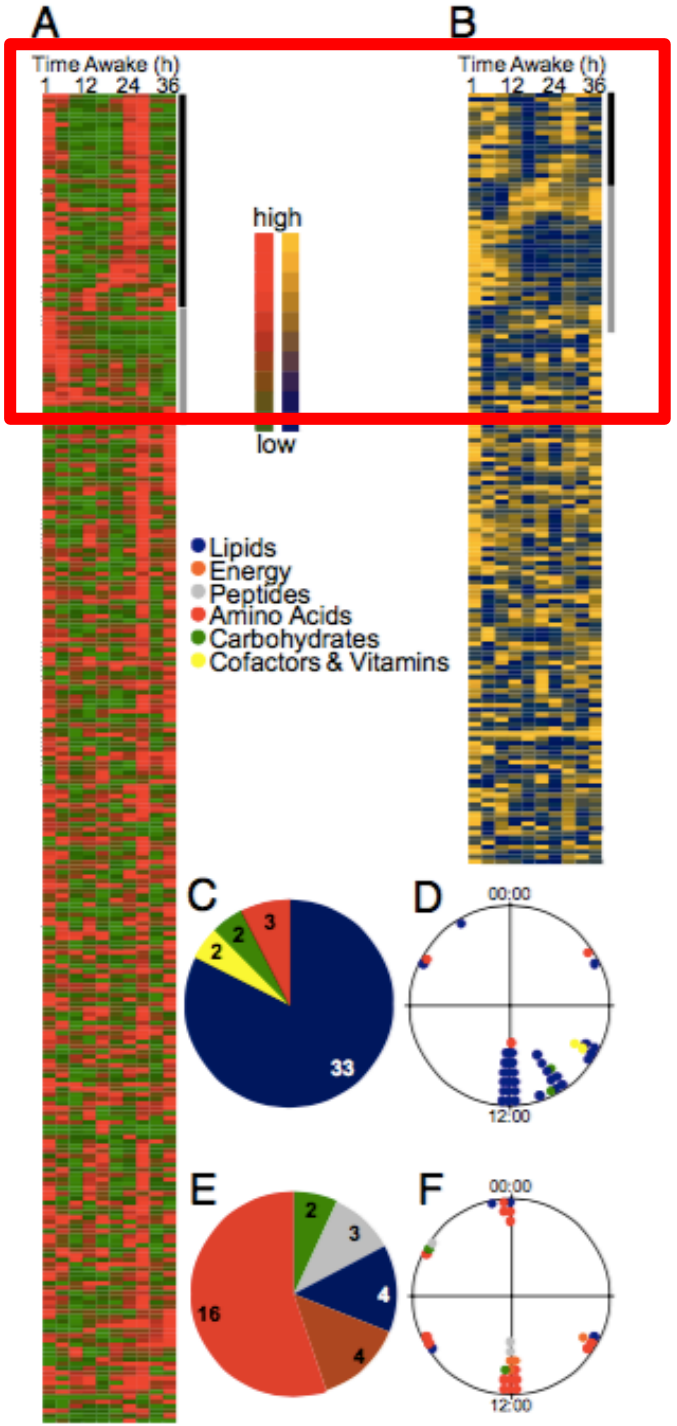
Arble et al, Obesity, 2009

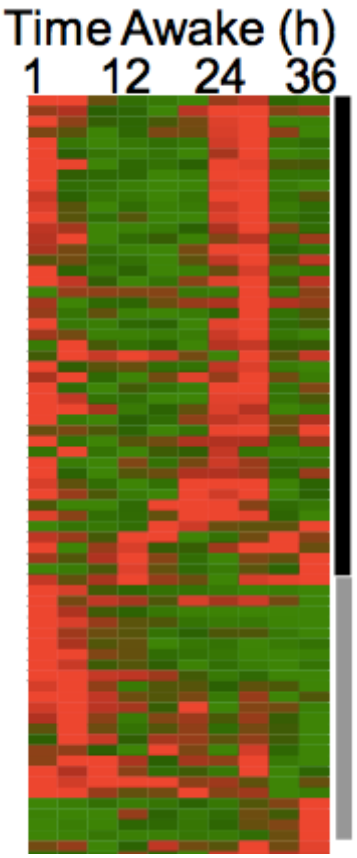
Weight-loss study in humans comparing late vs. early eaters.



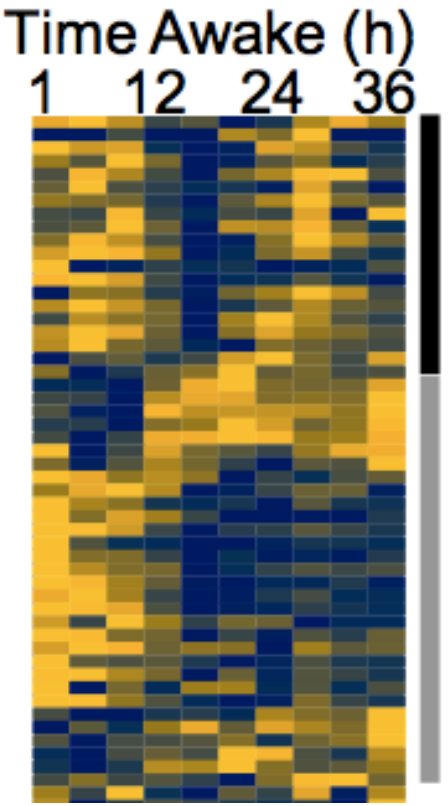
M Garaulet et al.,, *International Journal of Obesity* (2013)

Not just a sleep effect– human metabolism cycles as reflected in peripheral biofluids





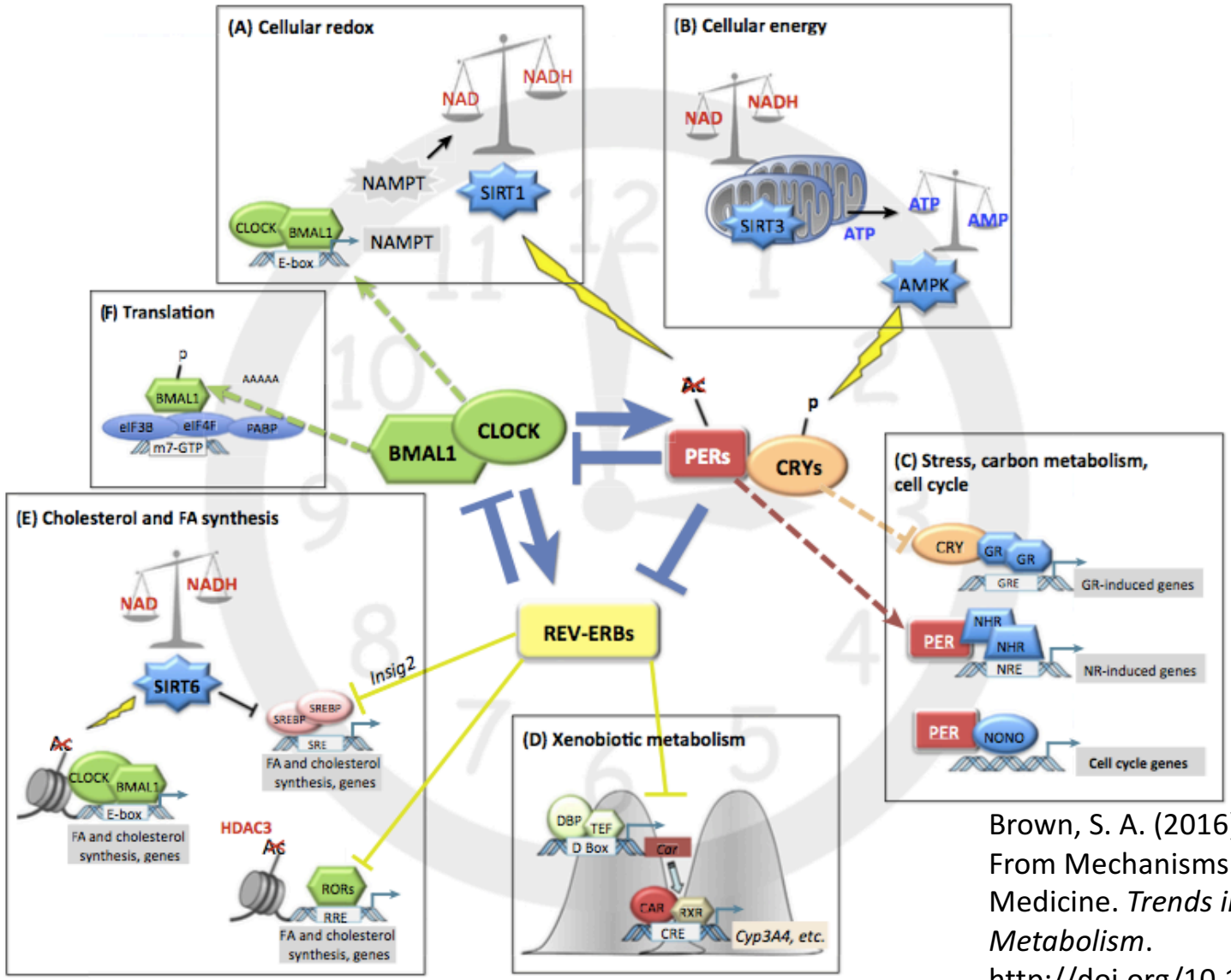
Plasma



Saliva

Dallman et al, PNAS, 2012

The core clock and metabolism are also connected at a molecular level through various transcriptional factors, nucear receptors and post-translational modifications

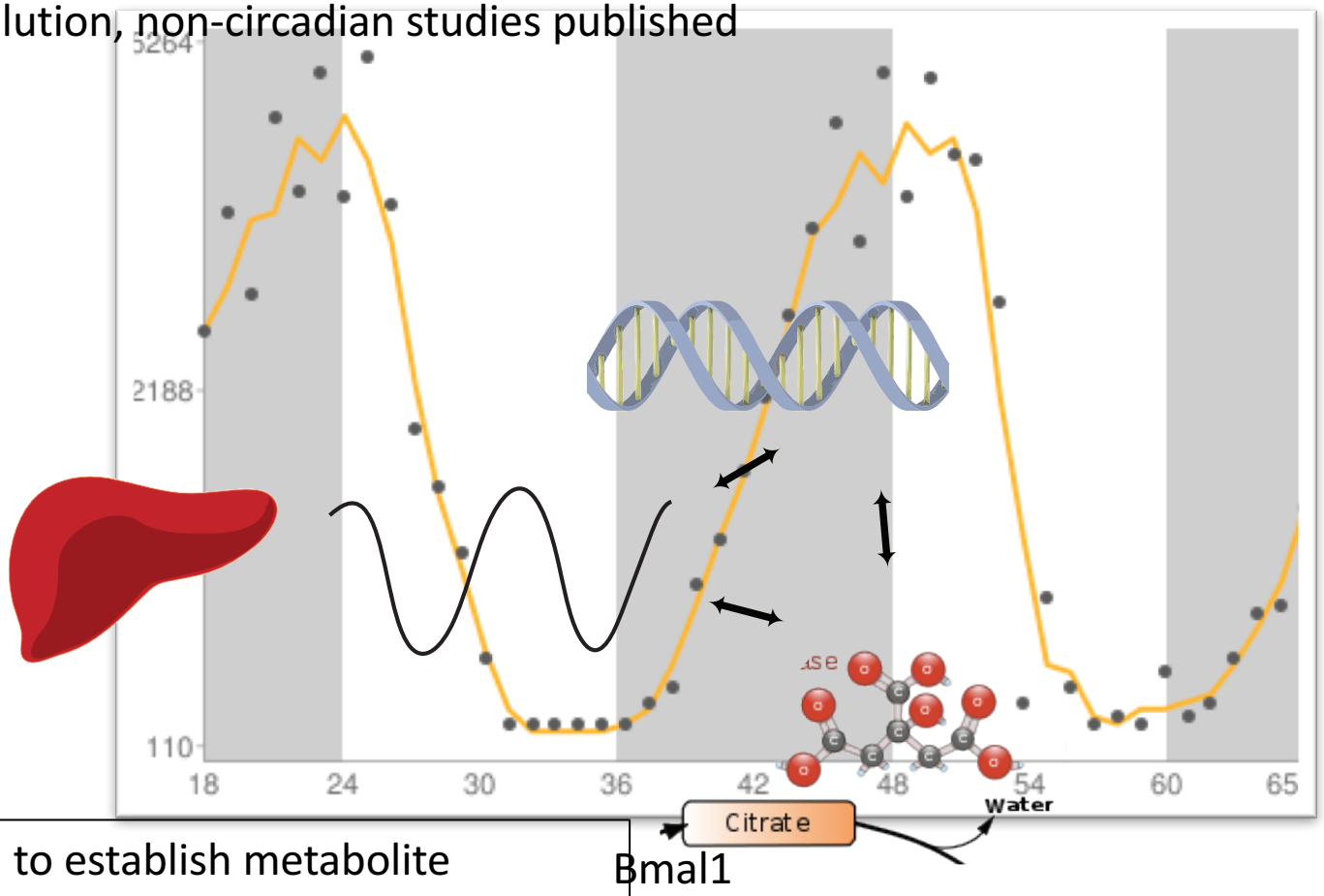


Brown, S. A. (2016). Circadian Metabolism: From Mechanisms to Metabolomics and Medicine. *Trends in Endocrinology & Metabolism*. http://doi.org/10.1016/j.tem.2016.03.015

Clock genes control transcription, but what is the nature of metabolite change in liver?

Some low-resolution, non-circadian studies published

Saikumari Krishnaiah

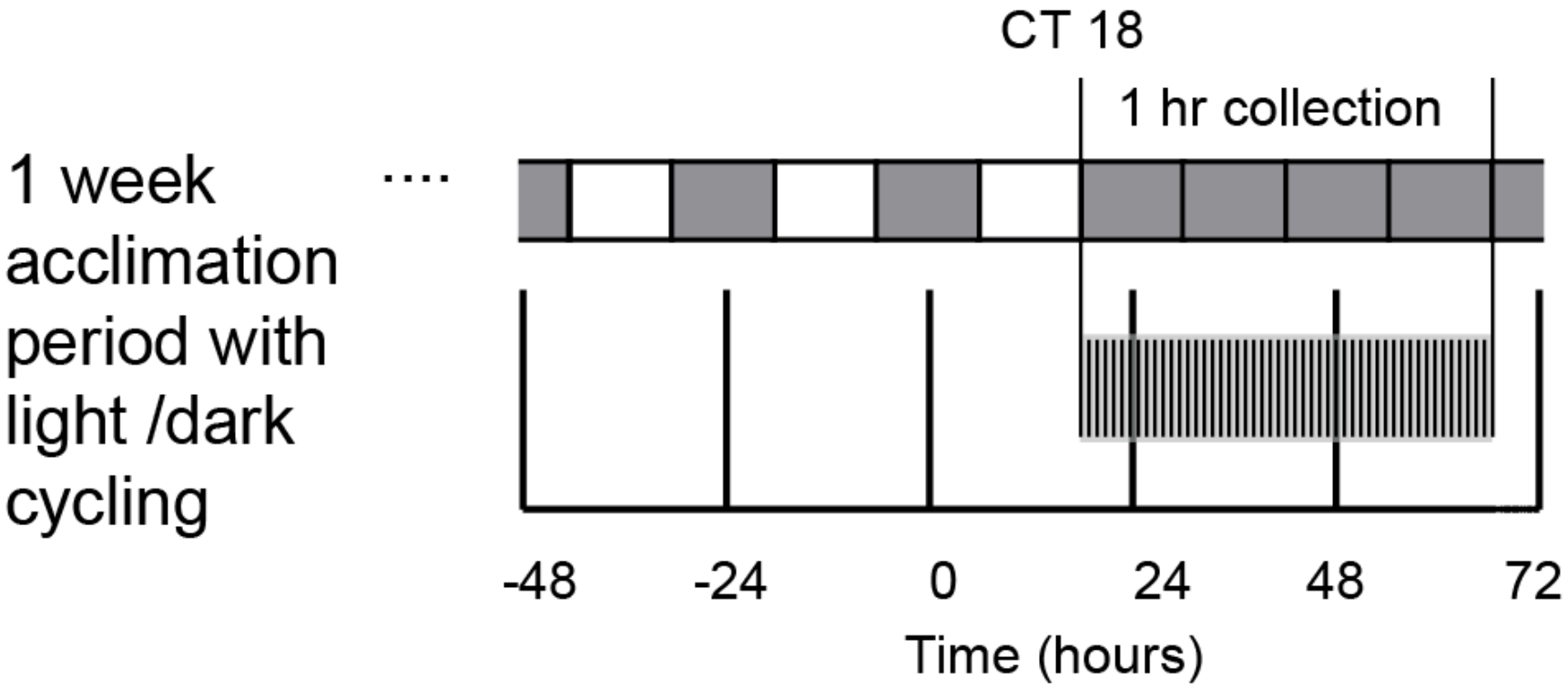


First we need to establish metabolite rhythmicity under true circadian conditions (dark / dark)

Sai Krishnaiah / John Hogenesch

Krishnaiah, et al. *Cell Metab* 2017

Samples were acquired every hour under circadian condition for 48 hours



Krishnaiah, et al. *Cell Metab* 2017

Workflow for both polar and non-polar metabolite analysis

Saikumari Krishnaiah

~400 named lipids +
>4000 lipid features

Sample

Cell pellet — Media

300ul of MeoH/CHCl3 (2:1)

FT /6times

sonicate

100ul of CHCl3/H2O (1:1)

Centrifuge for 7min/13300rpm

Organic

Internal stds added

dried in the hood

dissolved in solvent A and B

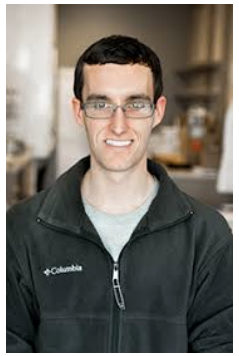
Xevo G2-S qTOF

Aqueous

speed vac/overnight

Re-dissolved in H_2O

Xevo TQD / TQS-micro



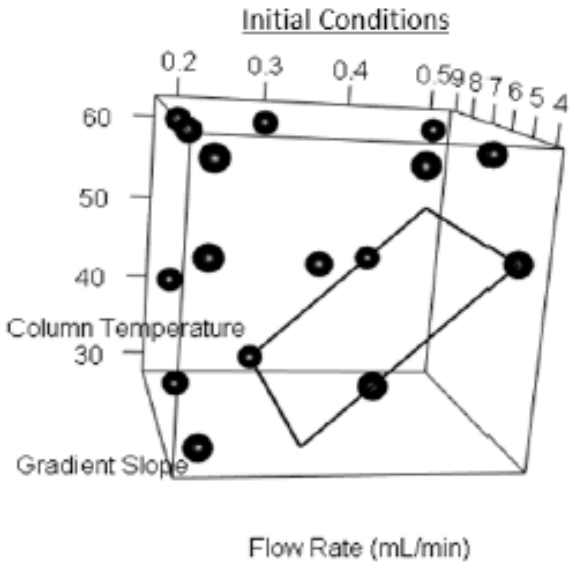
Seth Rhoades

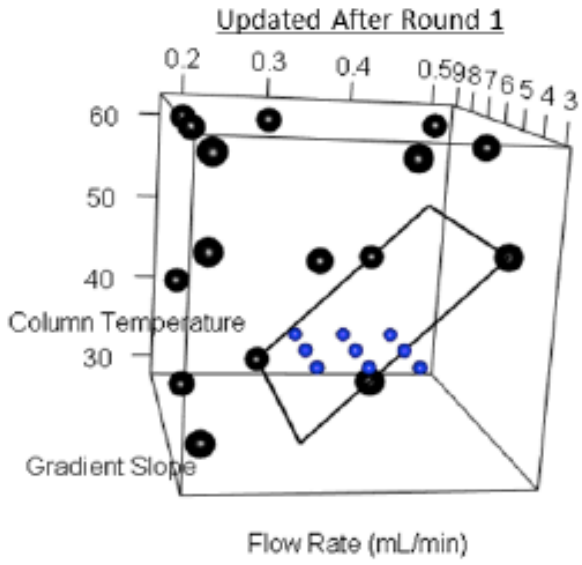
~340 metabolites targeted
Using COLMeD

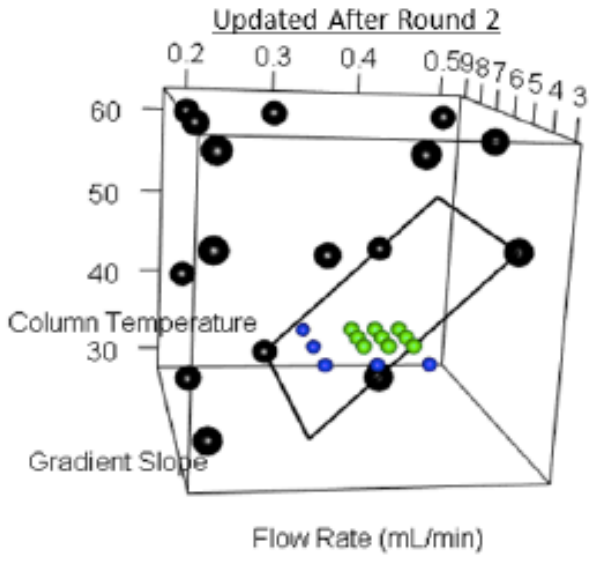
Applied cases:
Jang et al., Nat Med 2016
Tambellini et al, J. Prot. Res, 2017)

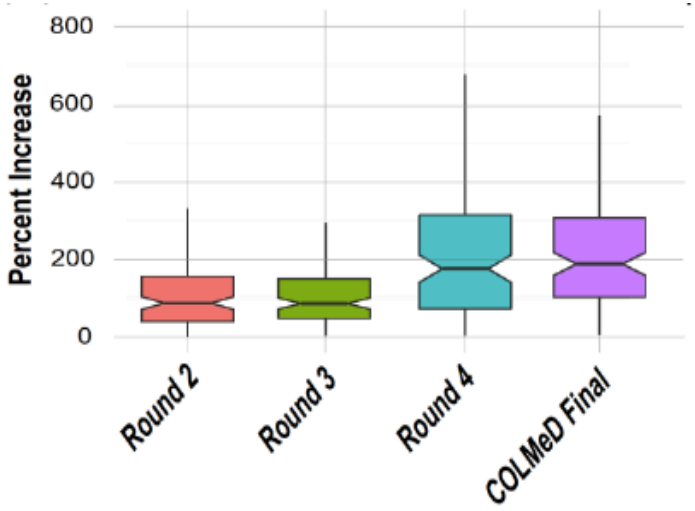
Rhoades & Weljie, Metabolomics, 2016

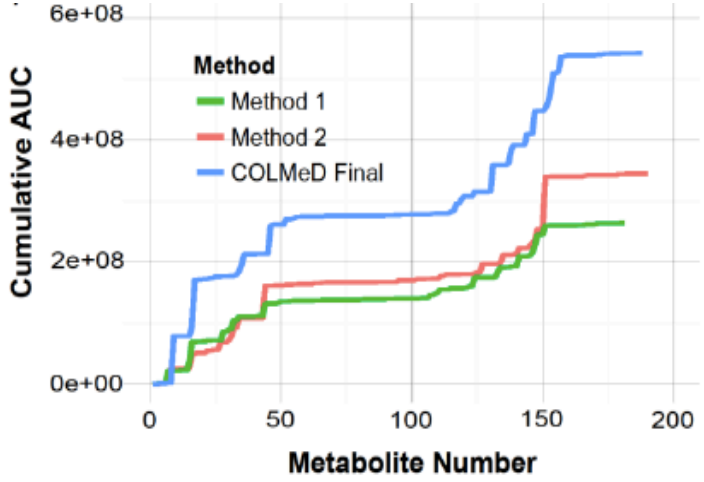
COLMeD - Comprehensive Optimization of LC-MS Metabolomics Methods Using Design of Experiments









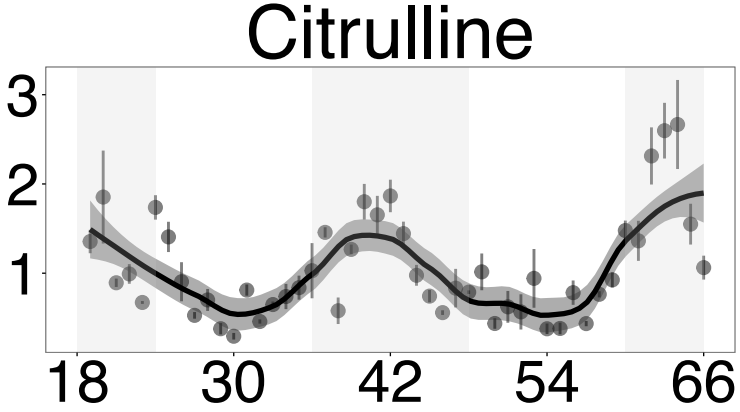


Rhoades & Weljie, Metabolomics, 2016

Overall metabolite cycling in liver is extremely robust

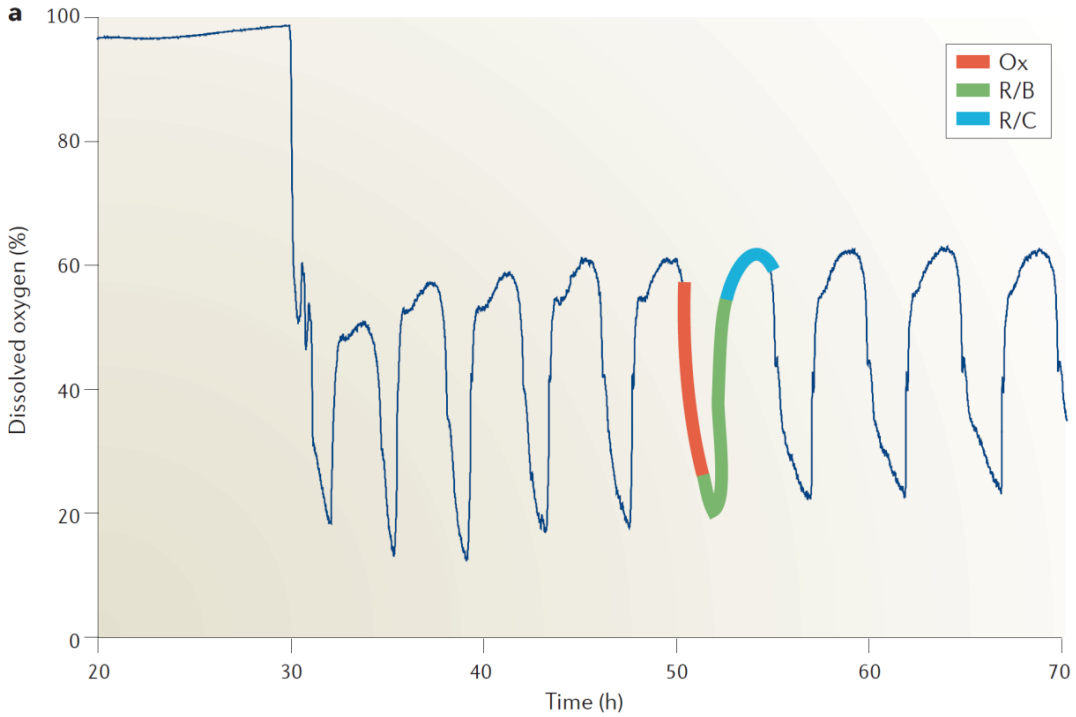
24 hour period tested

90 features (89/179) exhibit JTK BH.Q < 0.05 (~50%)



Krishnaiah, et al. *Cell Metab* 2017

Temporal compartmentalization of metabolism reduces futile cycling

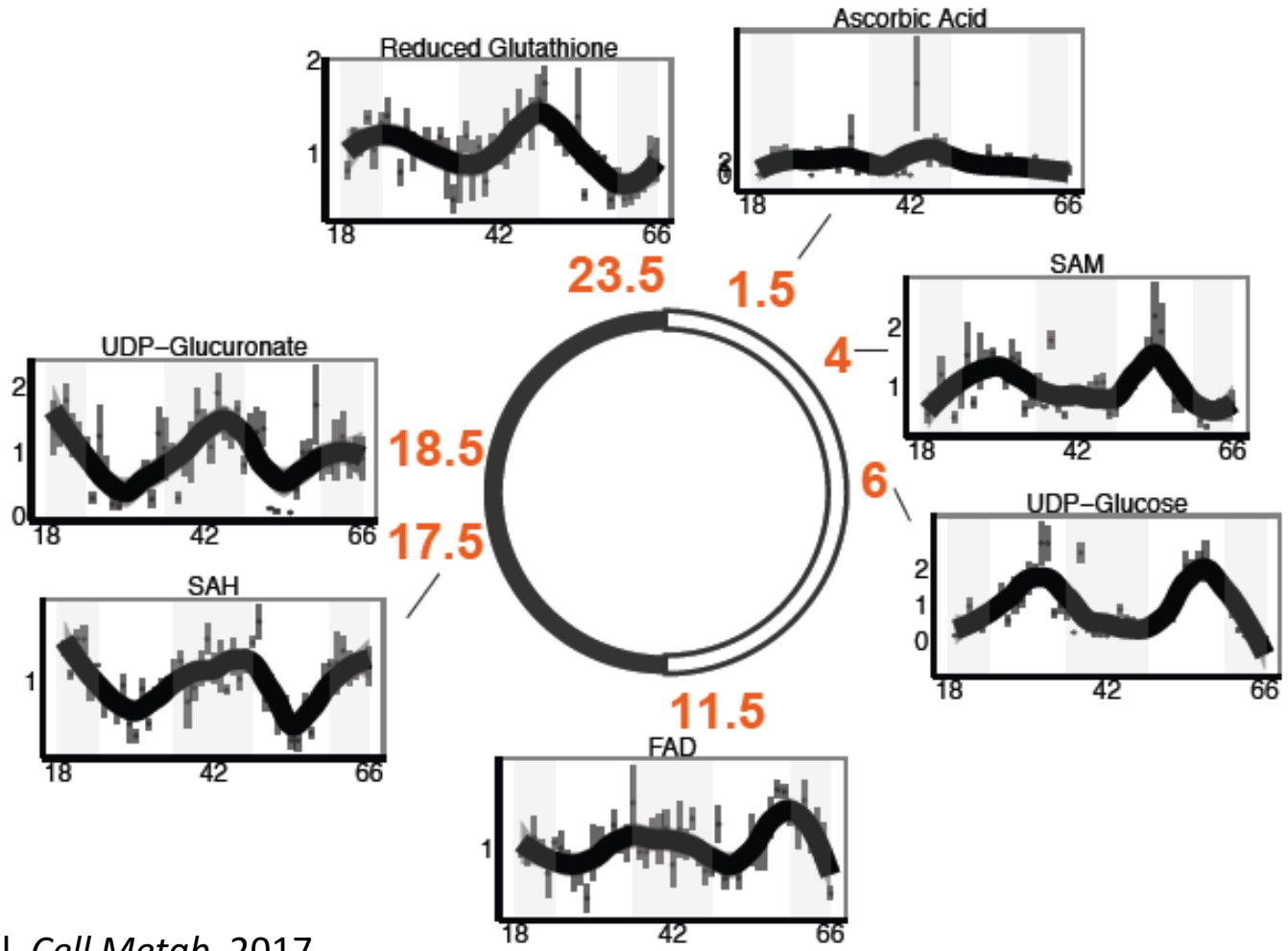


<u>Oxygen consumption in yeast</u>

- metabolic cycles of ~4–5 hours
- three major phases:
  - oxidative (Ox); reductive, building (R/B); reductive, charging (R/C)
- **Using time to segregate energetically incompatible reactions**

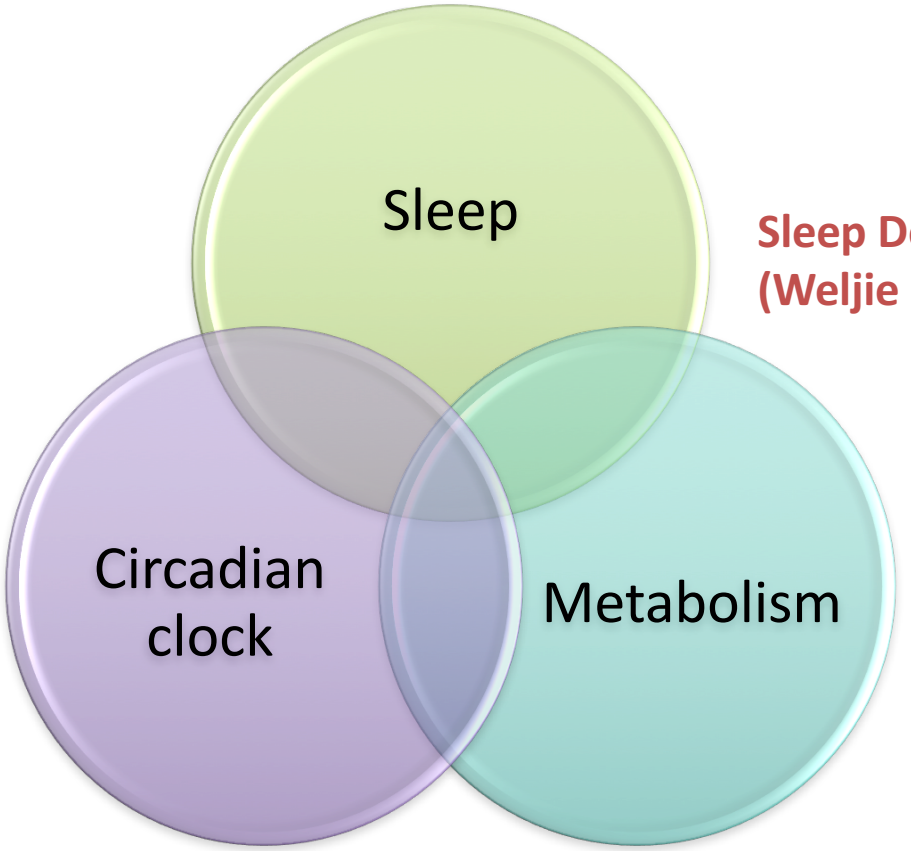
Tu BP & McKnight SL. Nat Rev Mol Cell Biol. 2006. PMID: 16823381

Methylation and redox processes strikingly temporally segregated



Krishnaiah, et al. *Cell Metab* 2017

Triad of sleep / circadian rhythms and metabolism increasingly implicated in disease processes

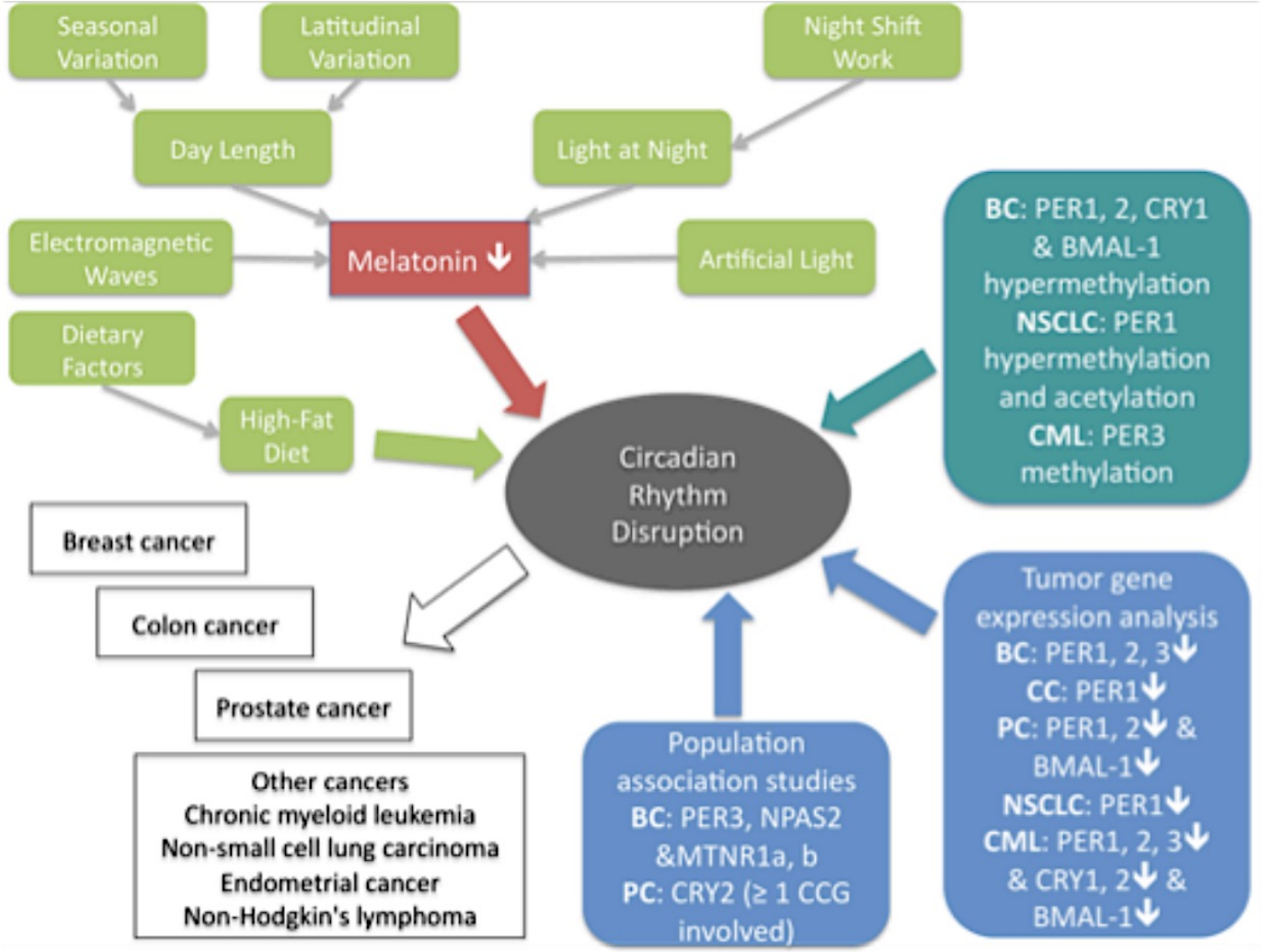


Sleep Deprivation
(Weljie et al, PNAS, 2015)

Lipids in cancer
(Krishnaiah et al, unpublished)
(Hao, Sengupta et al., unpublished)

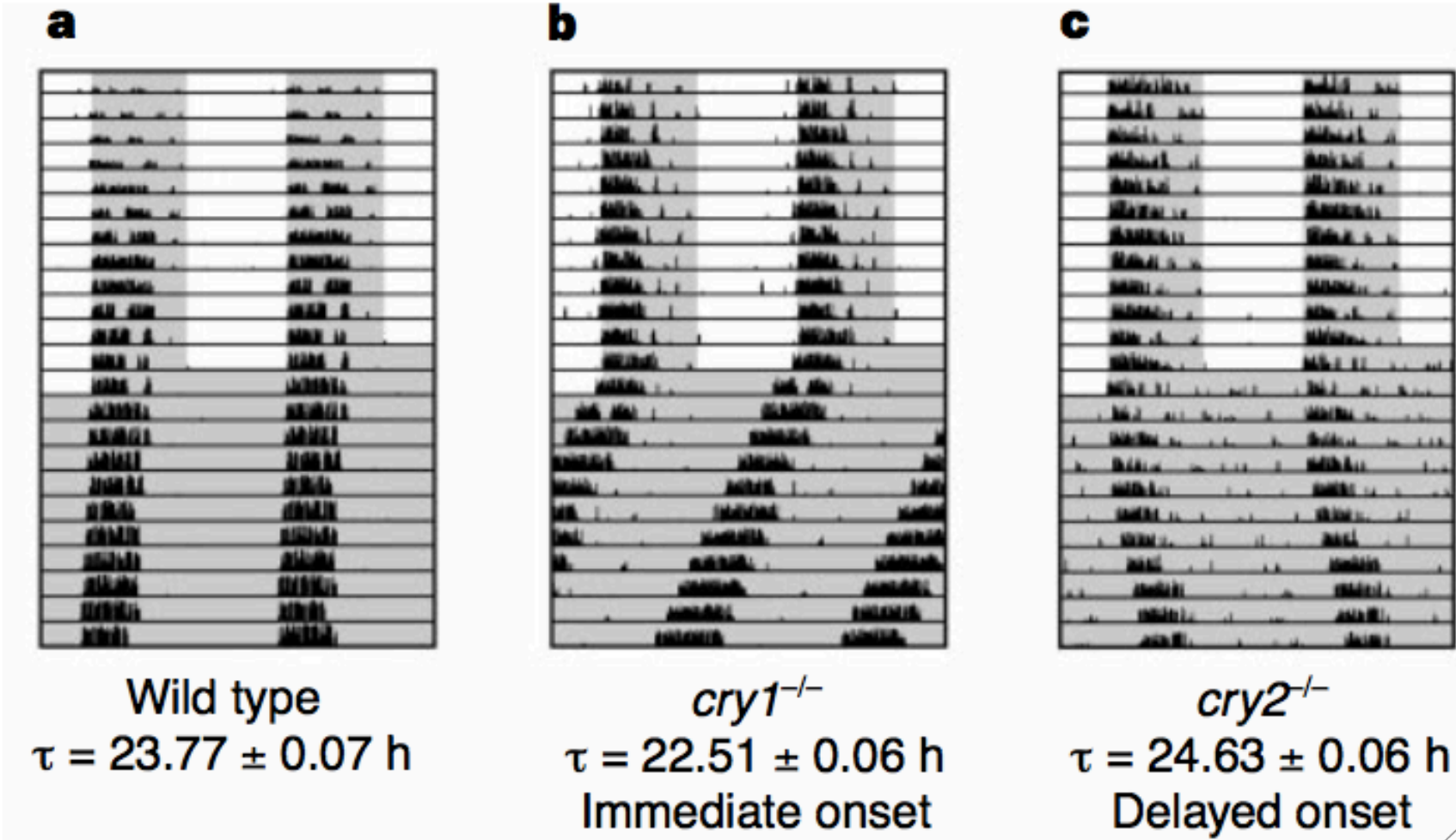
Clock-driven metabolism
(Krishnaiah et al, Cell Metabolism 2017)
(Skarke et al., Submitted)

Core clock genes are associated with cancer in humans



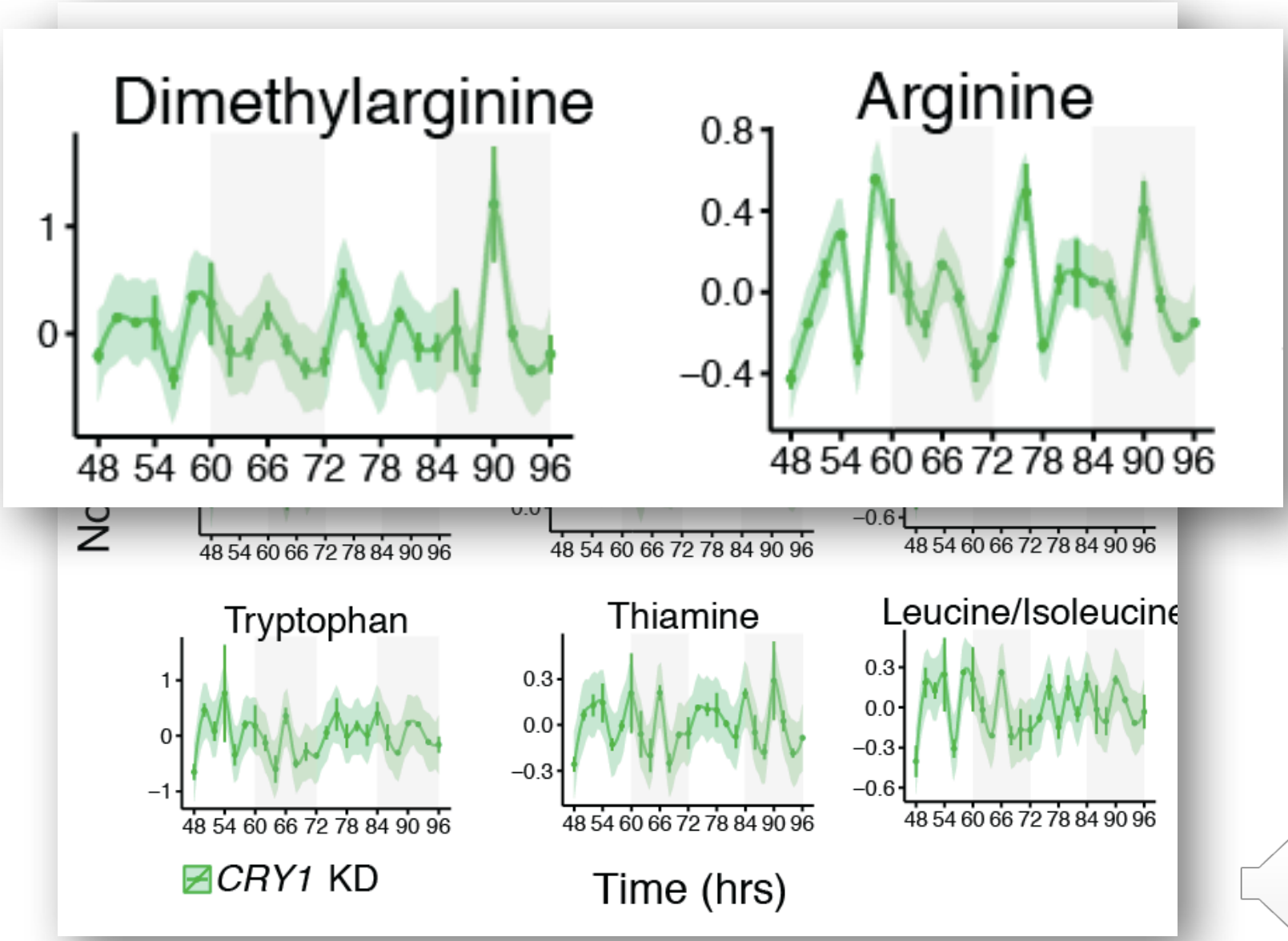
Sanmugam et al, J of Local and Global Health Science (2013)

Free-running mouse experiments under circadian conditions show cry1 loss shortens period and cry2 loss lengthens period



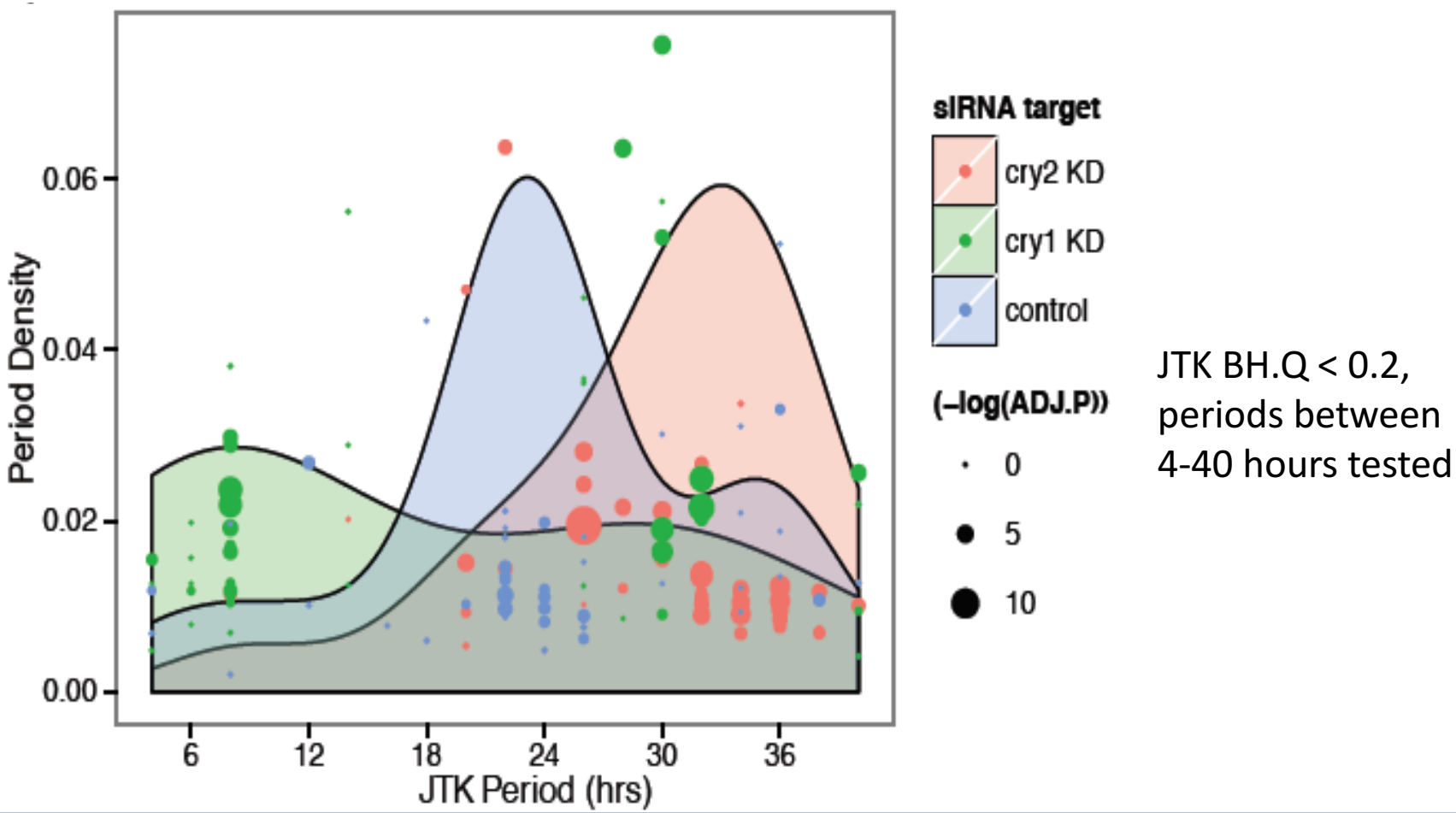
Hoeijmakers, J. H. J., Horst, G. T. J. V. D., Muijtjens, M., Kobayashi, K., Takano, R., Kanno, S.-I., et al. (1[illegible]9). Access : Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms : Nature. *Nature*, *398*(6728), 627–630. http://doi.org/10.1038/19323

Cry1 knockdown induces unexpected 8 h rhythms in metabolite cycling in a cell autonomous model!



Krishnaiah, et al. *Cell Metab* 2017

Cry1 and *Cry2* shift overall periods, although the cry1 shift is bimodal with one harmonic being extremely short (8 hrs)



JTK BH.Q < 0.2, periods between 4-40 hours tested

Cry 1 knock down in U2OS cells

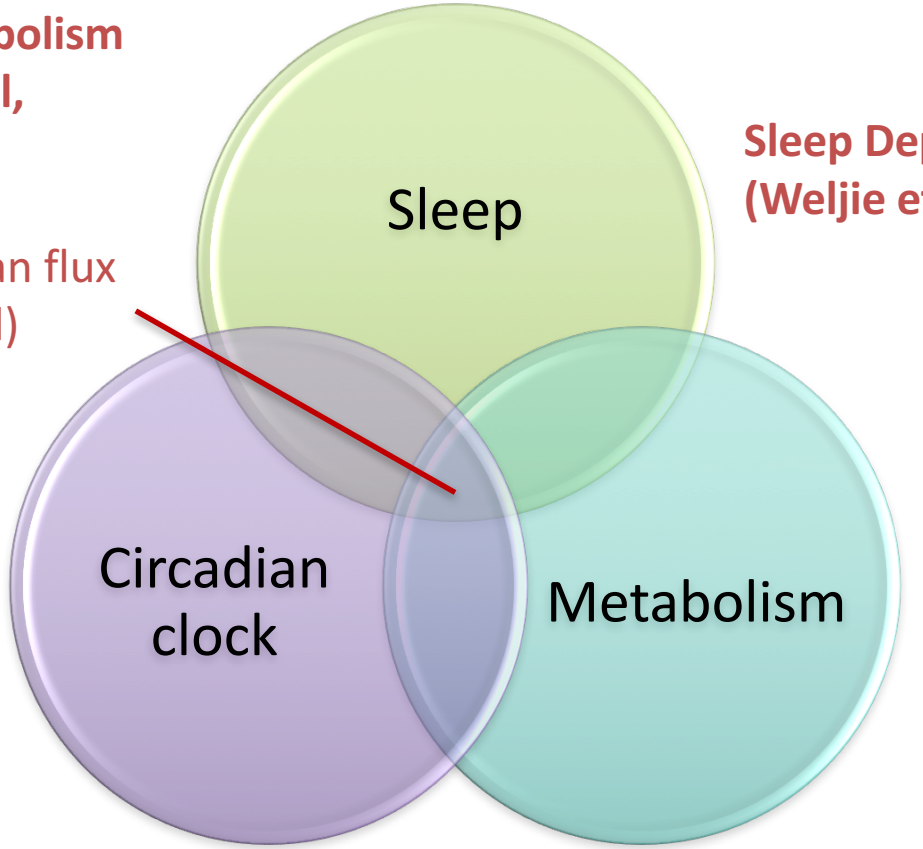
- Bimodal with one predominant harmonic being extremely short -8 h
- No 12 h harmonics compared to control
- These period shifts are greater than that observed in the transcriptional clock
- Cry 1 knock down leads to frequency decouple transcription from metabolite cycling.

Krishnaiah, et al. *Cell Metab* 2017

Triad of sleep / circadian rhythms and metabolism increasingly implicated in disease processes

Insomnia circadian metabolism (Gehrman, Sengupta et al, submitted)

Vistomic tracing / circadian flux (Rhoades et al, submitted)

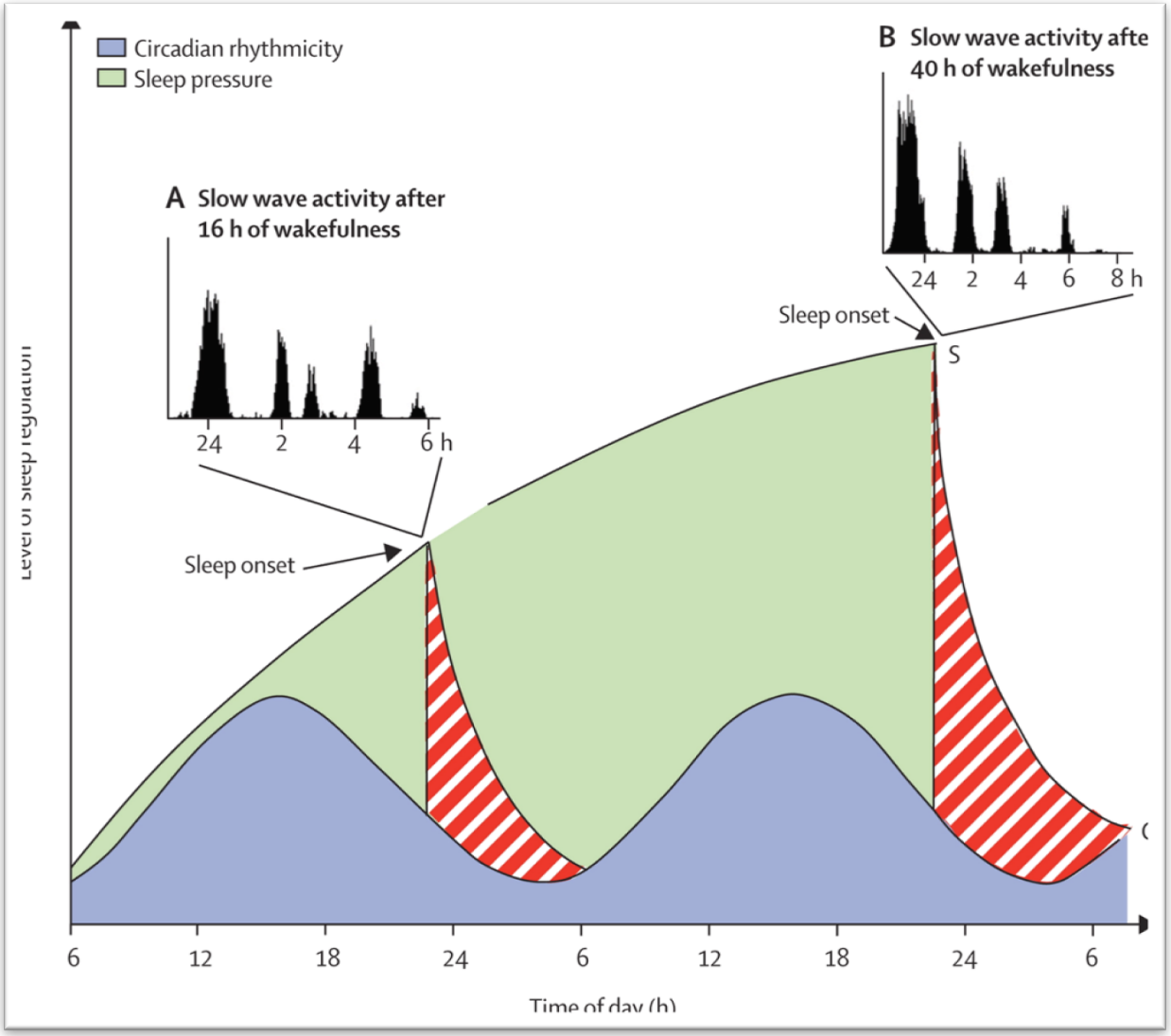


Sleep Deprivation (Weljie et al, PNAS, 2015)

Lipids in cancer (Krishnaiah et al, unpublished) (Hao, Sengupta et al., unpublished)

Clock-driven metabolism (Krishnaiah et al, Cell Metabolism 2017) (Skarke et al., Submitted)

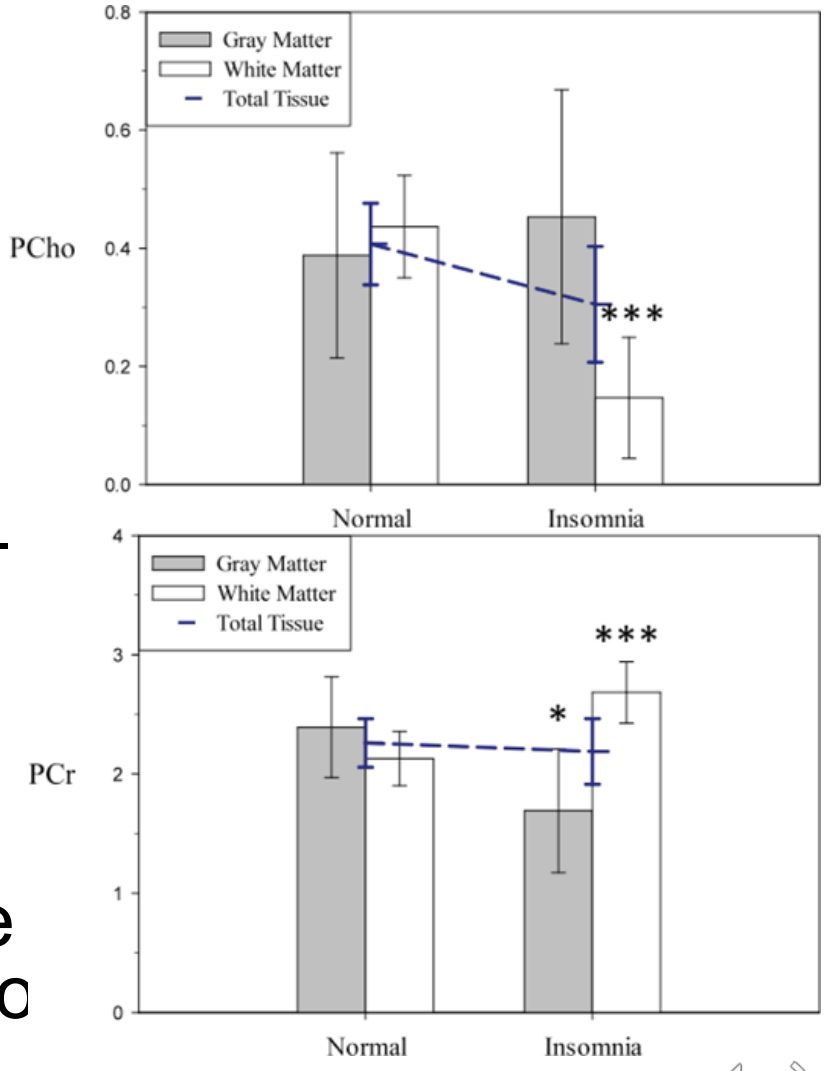
Sleep is regulated by a homeostatic and circadian process



Reimann et al, *The Lancet Neurology* 2015 14, 547-558DOI: (10.1016/S1474-4422(15)00021-6)

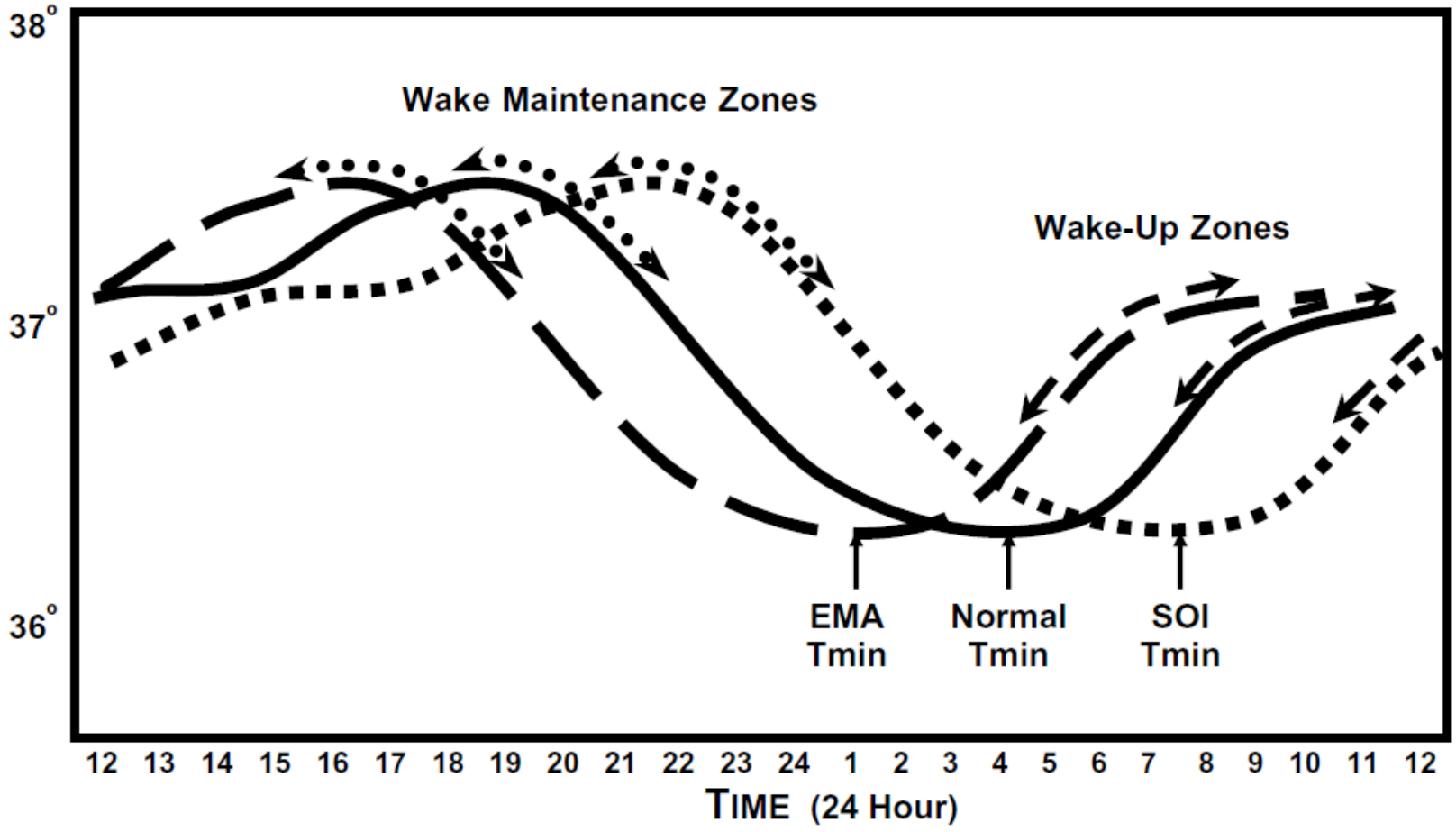
Insomnia as a disorder of hyperarsousal

- One of the top 10 causes of neuropsychiatric disability. (Source: NIMH)
- 10-15% of US population suffers from insomnia.
- Negative sequelae include – daytime fatigue, cognitive difficulty, impaired emotional regulation and overall decreased quality of life.
- Very little is known about the underlying pathophysiology o insomnia.



Harper et al, Sleep, 201

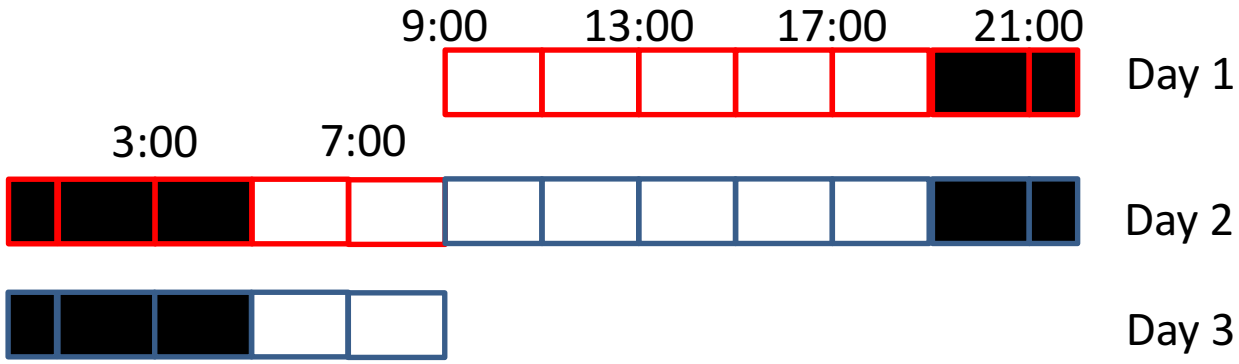
Insomnia has a circadian component; is there metabolic desynchrony?



EMA – early morning awakening
SOI – sleep onset insomniacs

Lack and Wright, 2007

Experimental design



N = 15 insomnia
15 controls
(age/sex matched)

Acclimatization timepoints
Sampling timepoints

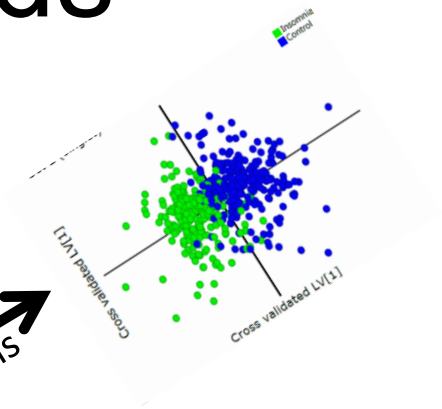
Overnight sleep study one night
-sleep stages
-sleep continuity

Blood samples withdrawn every 2 hours on both acclimatization and sampling time points. Blood serum was immediately recovered

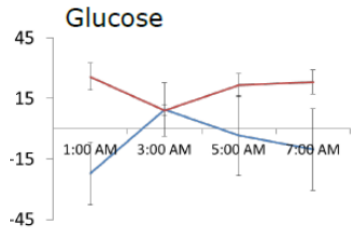
Metabolomic methods

1. Extraction by $MeOH/ChCl_3$
2. Polar fraction was subjected to further analysis

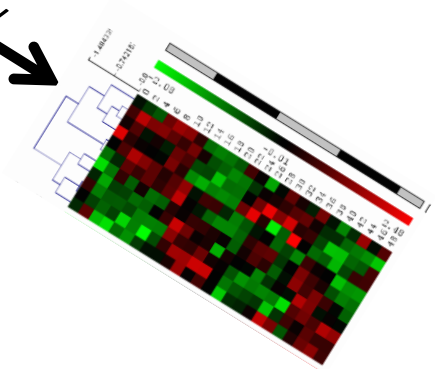
Multivariate Data analysis



Time series analysis

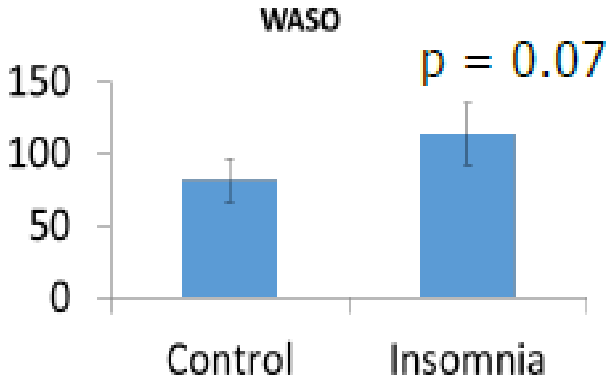


Rhythmicity analysis



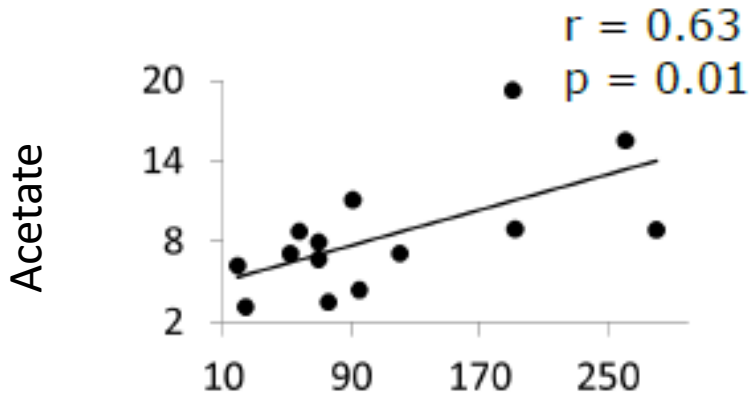
1. **Quantitative**
2. **Unbiased**
3. **Easy sample processing**
4. **Insensitive**

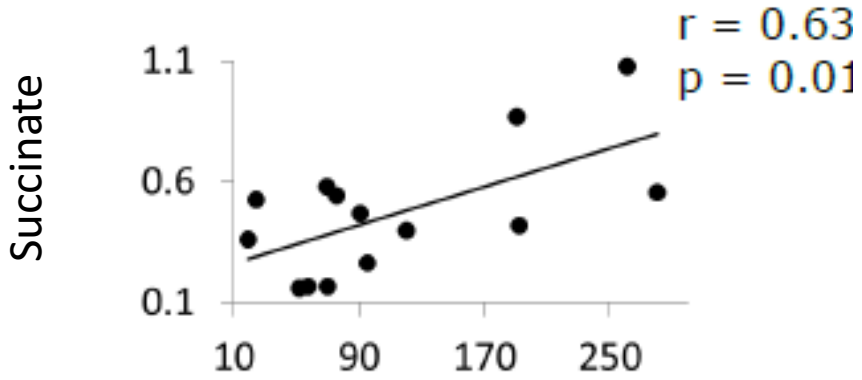
Metabolites are correlated with only wakefulness after sleep onset (WASO) amongst clinical variables



Morning levels of two energy metabolites are correlated significantly to the WASO scores of insomnia patients.

No such correlation was observed for controls.



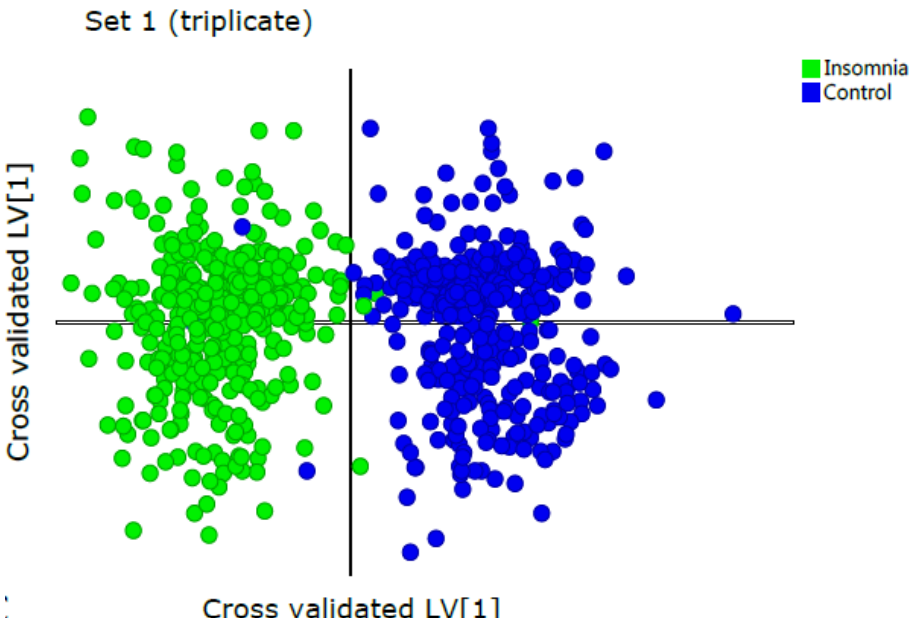


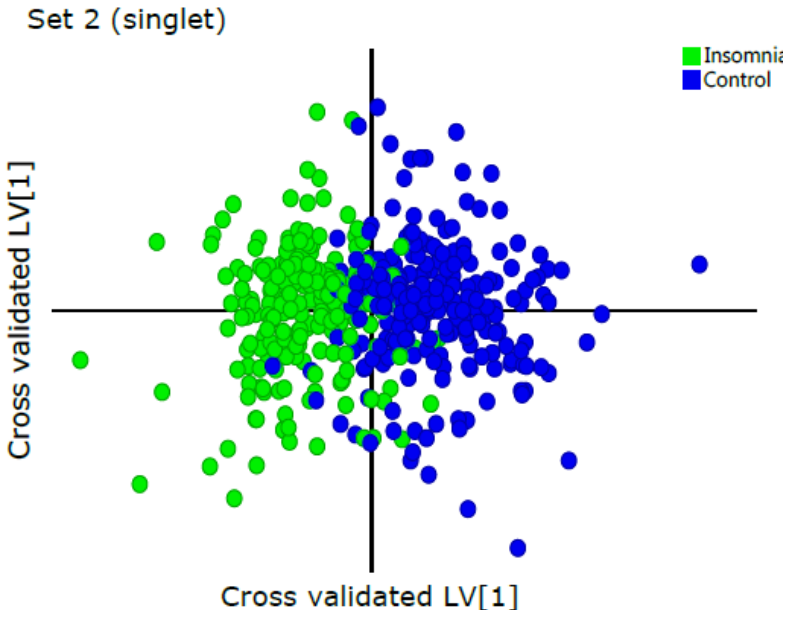
Hypothesis: Levels of circulatory metabolites may provide more information on insomnia than sleep architecture.

Global metabolic profiles of insomnia patients are distinct

Spectral analysis were performed on two distinct sets – 1. with analytical triplicates and 2. no analytical replicates.

Supervised multivariate OPLS-DA analysis

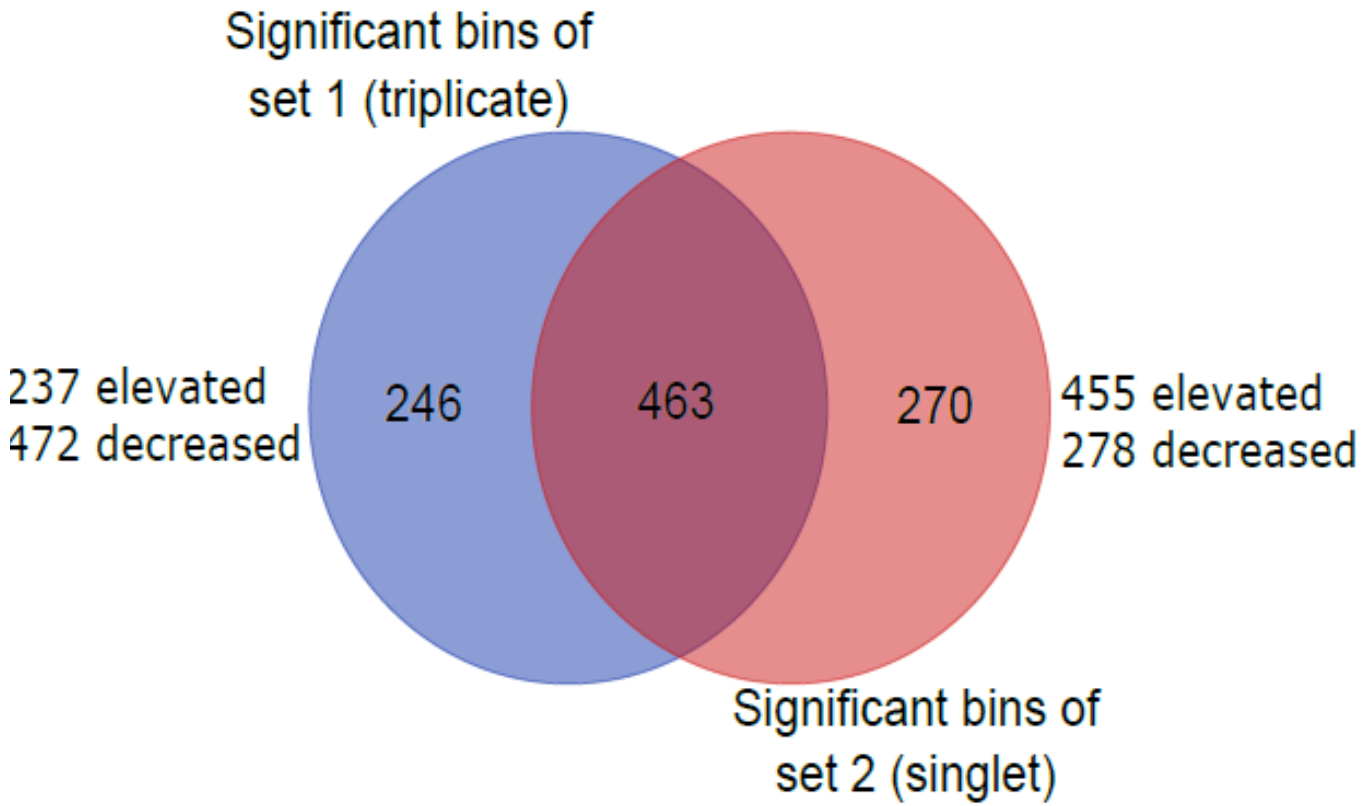




Q2 = 0.84
CV-ANOVA p<0.0001

Q2 = 0.58
CV-ANOVA p<0.0001

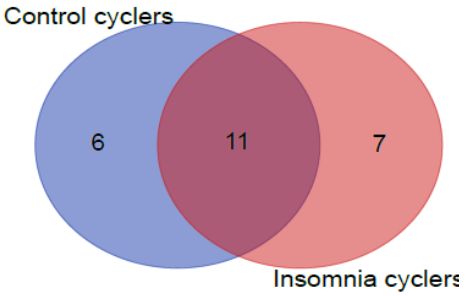
Global metabolic profiles of insomnia patients are distinct

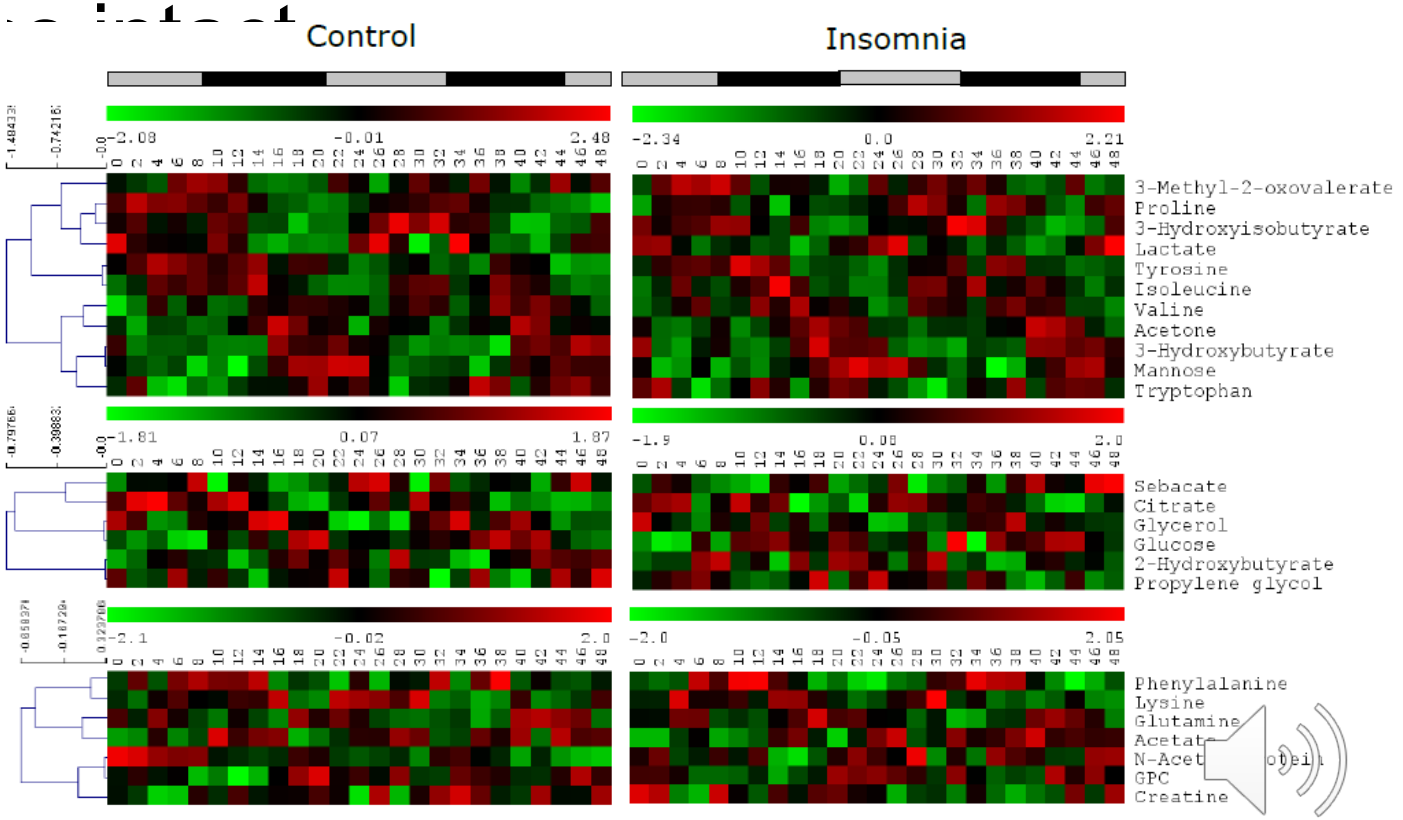


In this case, all time points from all the samples were considered. This will possibly obscure day / night differences

Insomnia affects metabolic oscillation

- Insomnia imparts new oscillatory metabolites while keeping most

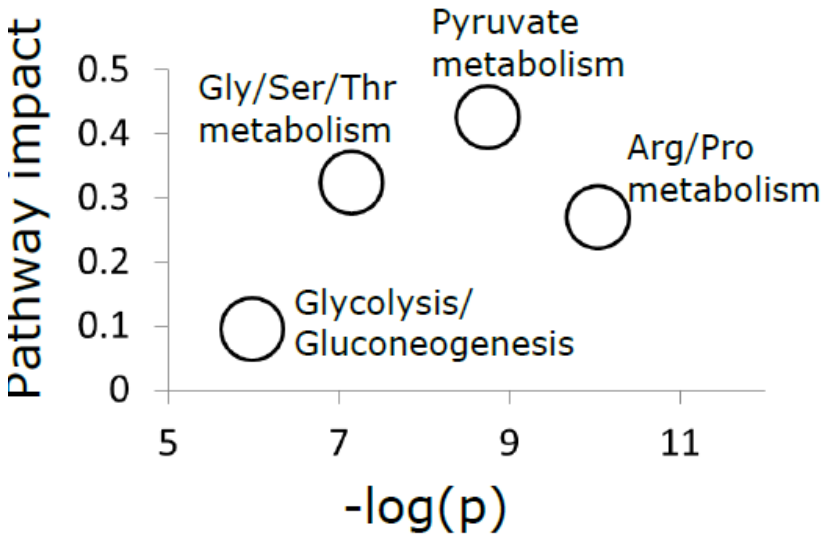


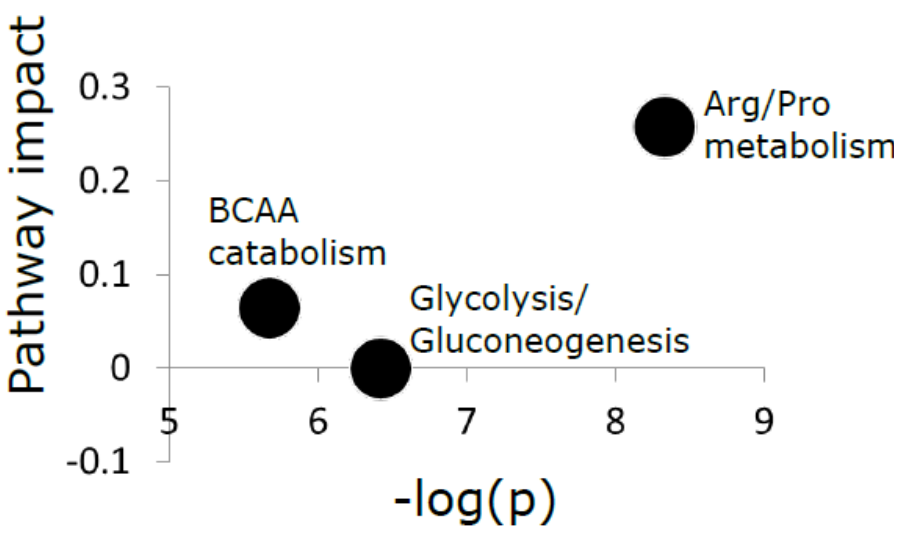


Control Cyclers

Insomnia Cyclers

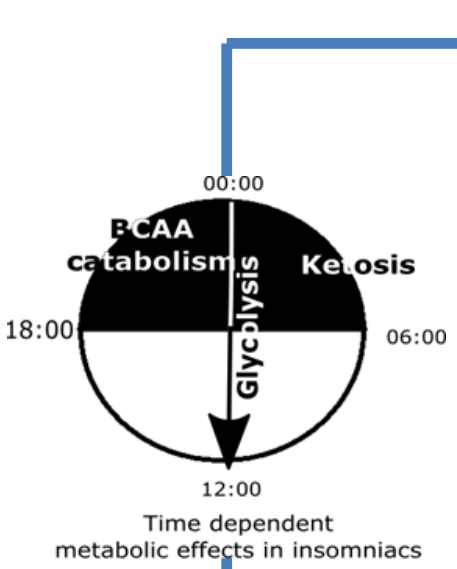
Pathway level metabolic alteration in insomnia patients





Metaboanalyst 3.0

Clinical implications of metabolic connection of insomnia



Time dependent metabolic effects in insomniacs

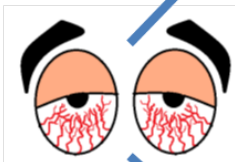
Glucose

Glycolysis

Pyruvate

TCA Cycle

Hyperarousal
1. Higher energy need
2. Elevated catabolism



Objective insomnia quantification

Acetate
Succinate

Diabetes?

Perturbed body clock
1. Temporal loss of anabolism-catabolism balance.
2. More catabolic activity during bedtime.

BCAA catabolism

Epidemiology/Health Services Research
ORIGINAL ARTICLE

Insomnia With Objective Short Sleep Duration Is Associated With Type 2 Diabetes

A population-based study

Broad range of sleep disorders– many opportunities for metabolic study

Discussion points– Impact of metabolomics

- How deep do we need to go in Global Metabolomics analysis?
  - Are platform biases a long-term hindrance?
- Systemic metabolite biomarkers of circadian / sleep disruption to mechanistic interpretation– how useful are translational models?
  - Challenges of nocturnal vs diurnal species, photic response differences, sleep architecture, metabolic capacity
  - Are sleep metabolic changes observed circadian or due to metabolic homeostasis?
  - Human variability!
- Relevance of ultradian oscillations in cell-autonomous systems in a clinical context?
  - Disruption of ordered temporal segregation favorable for disease?

Acknowledgements

- Weljie Lab
  - Saikumari Krishnaiah
  - Seth Rhoades
  - Arjun Sengupta
  - Dania Malik
  - Ubeydullah ER
  - Bahar Sianati
  - Sidney Bolden
  - Barry Slaff
- Amita Sehgal
- Peter Meerlo (Groningen)
- Ted Abel (Iowa)
- David Dinges
- Namni Goel
- Matt Kayser
- Morrie Birinbaum (Pfizer)
- John Hogenesch (Cincinnati)
  - Jacki Growe
  - Lauren Francey
  - Anand Venkataraman
  - Anthony Olarerin-George
- Chi Van Dang (Wistar)
  - Brian Altman
- Aziz Sancar (UNC)
  - Chris Selby
- Ron Anafi
- I. Halil Kavakli (Koc U., Turkey)
  - Sibel Cal
- Phil Gehrman
- Garret Fitzgerald
- Carsten Skarke
- Casey Greene