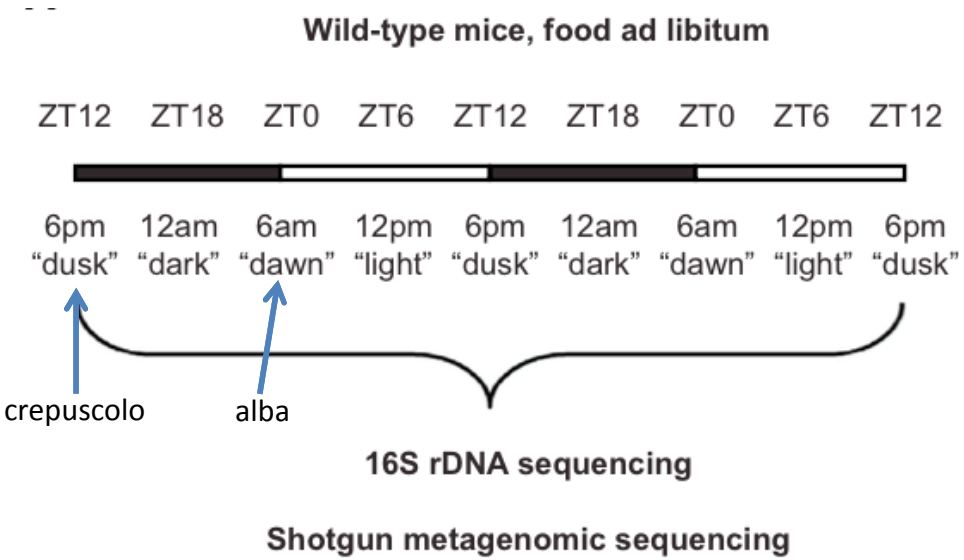
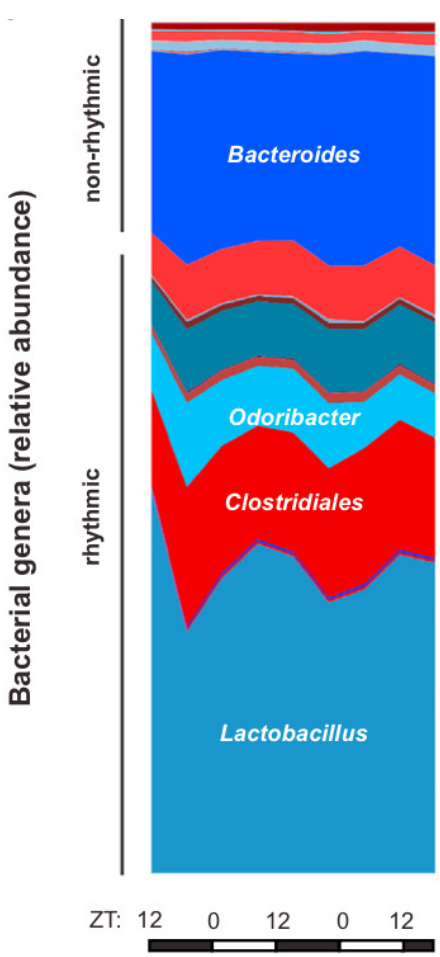
Cell Ottobre 2014

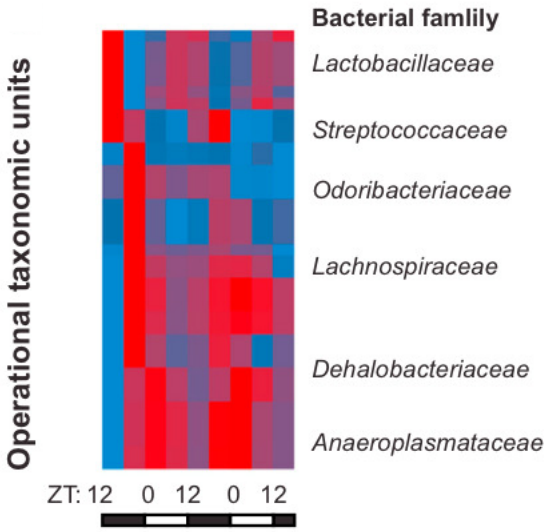
Transkingdom Control of Microbiota Diurnal Oscillations Promotes Metabolic Homeostasis

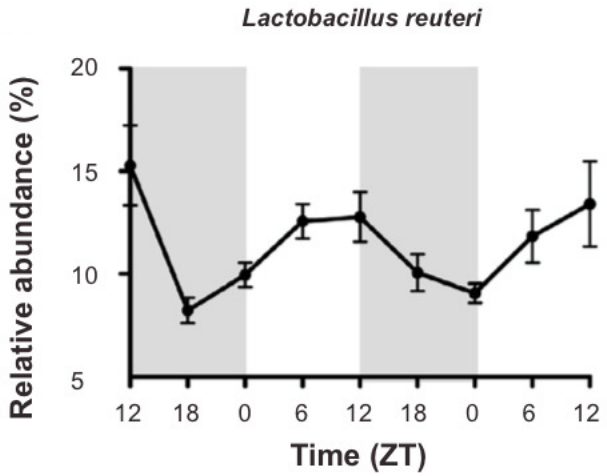
Christoph A. Thaiss,[1] David Zeevi,[2] Maayan Levy,[1] Gili Zilberman-Schapira,[1] Jotham Suez,[1] Anouk C. Tengeler,[1] Lior Abramson,[1] Meirav N. Katz,[1,3] Tal Korem,[2] Niv Zmora,[3,4,5] Yael Kuperman,[6] Inbal Biton,[6] Shlomit Gilad,[7] Alon Harmelin,[6] Hagit Shapiro,[1] Zamir Halpern,[3,5] Eran Segal,[2] and Eran Elinav[1,*]

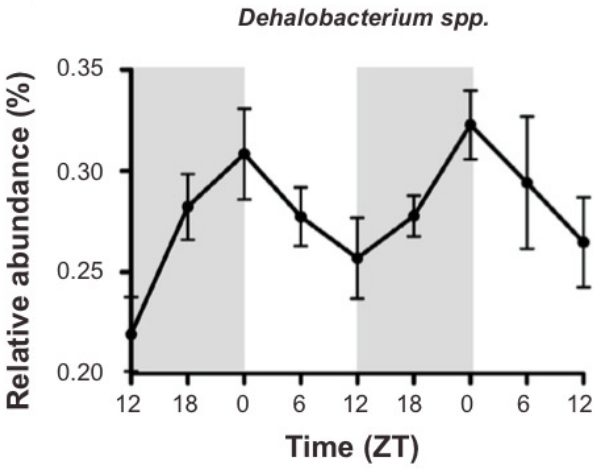


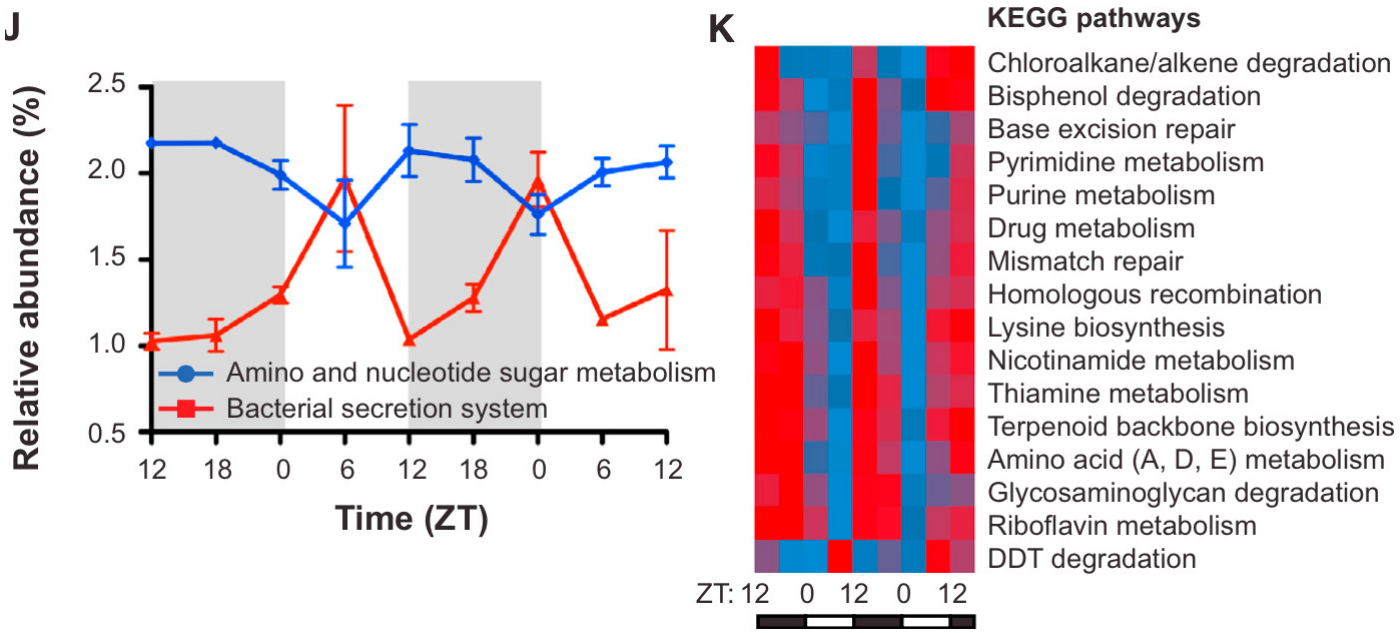
In humans, disruption of the circadian clock is a common hallmark of the modern alteration in lifestyle and is especially evident in individuals engaged in chronic shift work or frequently flying across time zones and experiencing the "jet lag" phenomenon. This new set of disruptive conditions to human physiology is associated with a propensity for a wide range of diseases, including obesity, diabetes, cancer, cardiovascular disease, and susceptibility to infection (Archer et al., 2014; Buxton et al., 2012; Fonken et al., 2010; Scheer et al., 2009; Suwazono et al., 2008). The mechanisms by which disruption of circadian rhythmicity contributes to these pathophysiological outcomes remain largely unknown.


Bacterial genera (relative abundance)
non-rhythmic
rhythmic
Bacteroides
Odoribacter
Clostridiales
Lactobacillus
ZT: 12 0 12 0 12

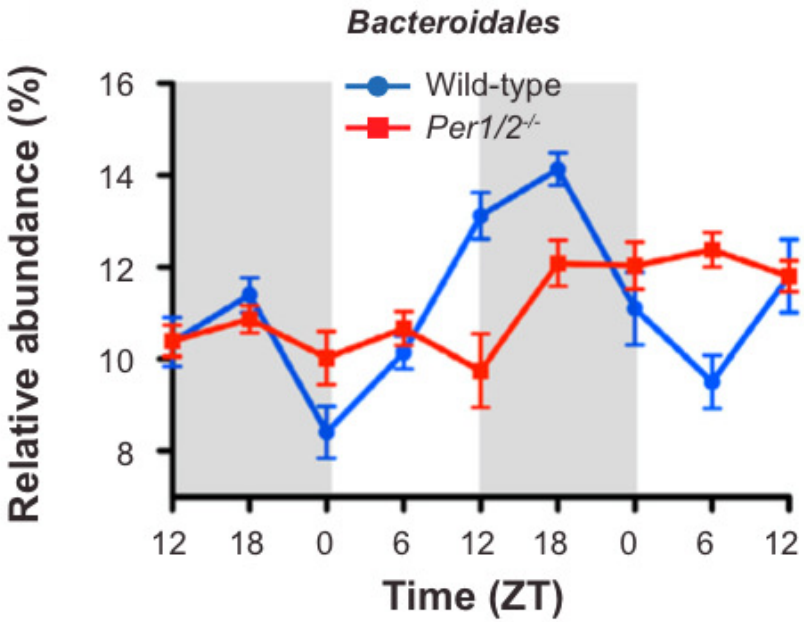

Operational taxonomic units
Bacterial famlily
Lactobacillaceae
Streptococcaceae
Odoribacteriaceae
Lachnospiraceae
Dehalobacteriaceae
Anaeroplasmataceae
ZT: 12 0 12 0 12


Lactobacillus reuteri
Relative abundance (%)
20
15
10
5
12 18 0 6 12 18 0 6 12
Time (ZT)

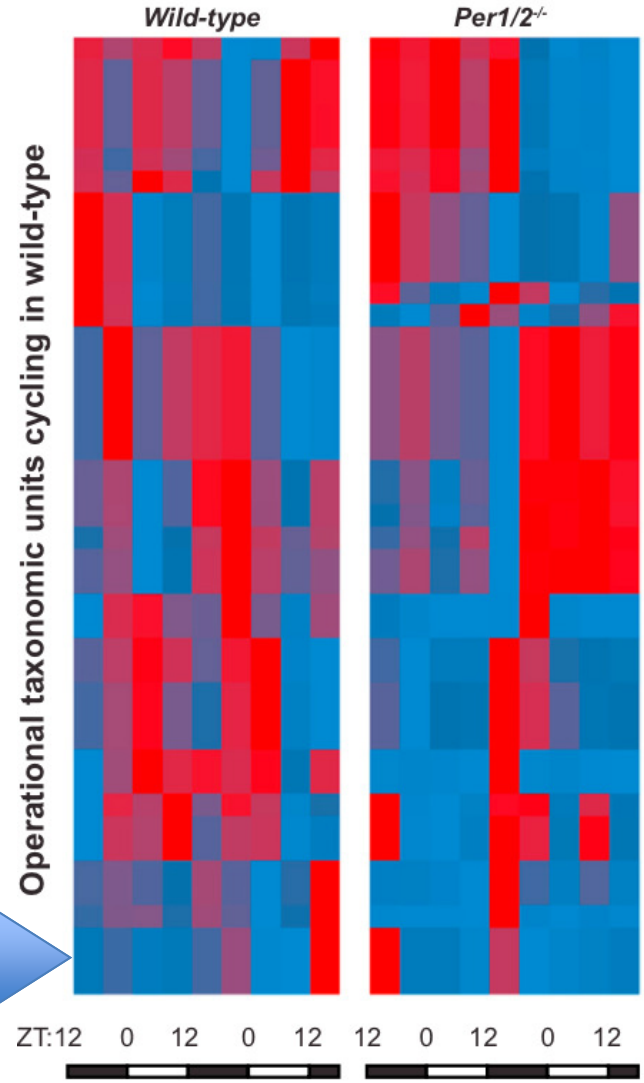

Dehalobacterium spp.
Relative abundance (%)
0.35
0.30
0.25
0.20
12 18 0 6 12 18 0 6 12
Time (ZT)

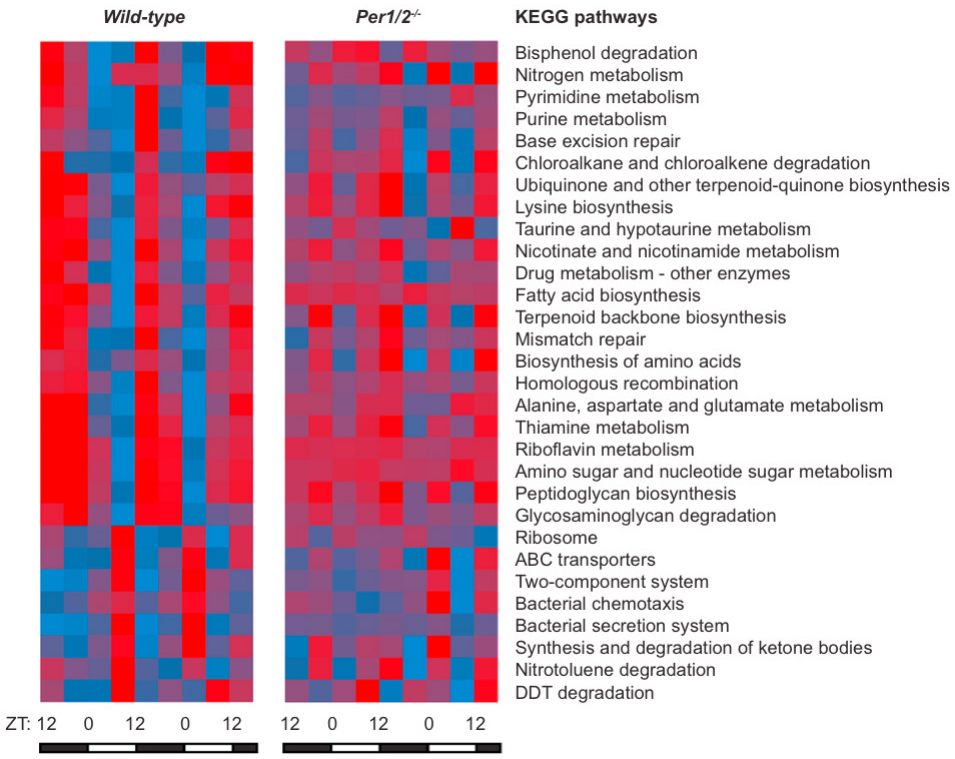

J
Relative abundance (%)
2.5
2.0
1.5
1.0
0.5
12
18
0
6
12
18
0
6
12
Time (ZT)
Amino and nucleotide sugar metabolism
Bacterial secretion system
K
KEGG pathways
Chloroalkane/alkene degradation
Bisphenol degradation
Base excision repair
Pyrimidine metabolism
Purine metabolism
Drug metabolism
Mismatch repair
Homologous recombination
Lysine biosynthesis
Nicotinamide metabolism
Thiamine metabolism
Terpenoid backbone biosynthesis
Amino acid (A, D, E) metabolism
Glycosaminoglycan degradation
Riboflavin metabolism
DDT degradation
ZT: 12 0 12 0 12

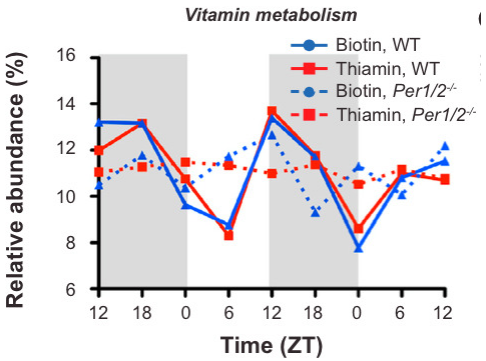
Per1/2$^{-/-}$ deficient in a functional host clock

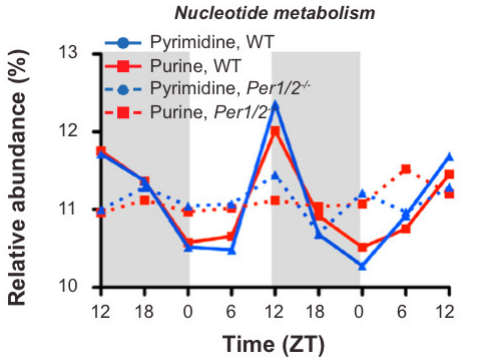


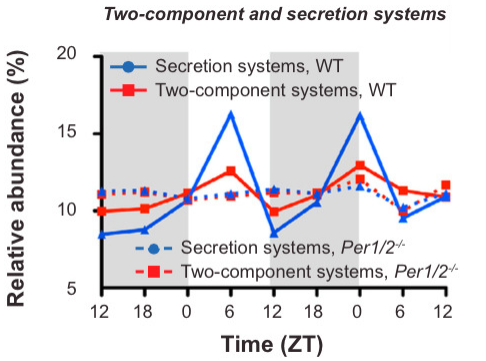
The rhythmic pattern observed in wild-type mice was replaced by a random abundance fluctuation in clock-deficient mice with a reduction in the number of diurnally oscillating bacterial taxonomic units




Wild-type
Per1/2-/-
KEGG pathways
Bisphenol degradation
Nitrogen metabolism
Pyrimidine metabolism
Purine metabolism
Base excision repair
Chloroalkane and chloroalkene degradation
Ubiquinone and other terpenoid-quinone biosynthesis
Lysine biosynthesis
Taurine and hypotaurine metabolism
Nicotinate and nicotinamide metabolism
Drug metabolism - other enzymes
Fatty acid biosynthesis
Terpenoid backbone biosynthesis
Mismatch repair
Biosynthesis of amino acids
Homologous recombination
Alanine, aspartate and glutamate metabolism
Thiamine metabolism
Riboflavin metabolism
Amino sugar and nucleotide sugar metabolism
Peptidoglycan biosynthesis
Glycosaminoglycan degradation
Ribosome
ABC transporters
Two-component system
Bacterial chemotaxis
Bacterial secretion system
Synthesis and degradation of ketone bodies
Nitrotoluene degradation
DDT degradation
ZT: 12 0 12 0 12
12 0 12 0 12


Vitamin metabolism
Biotin, WT
Thiamin, WT
Biotin, Per1/2-/-
Thiamin, Per1/2-/-
Relative abundance (%)
16
14
12
10
8
6
12 18 0 6 12 18 0 6 12
Time (ZT)

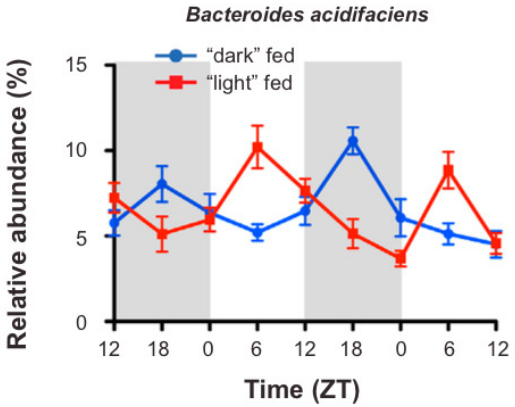

Nucleotide metabolism
Pyrimidine, WT
Purine, WT
Pyrimidine, Per1/2-/-
Purine, Per1/2-/-
Relative abundance (%)
13
12
11
10
12 18 0 6 12 18 0 6 12
Time (ZT)

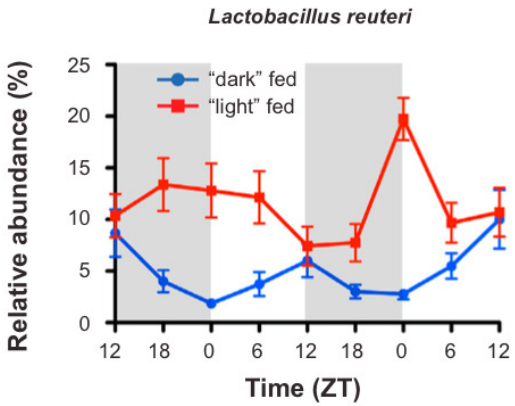

Two-component and secretion systems
Secretion systems, WT
Two-component systems, WT
Secretion systems, Per1/2-/-
Two-component systems, Per1/2-/-
Relative abundance (%)
20
15
10
5
12 18 0 6 12 18 0 6 12
Time (ZT)

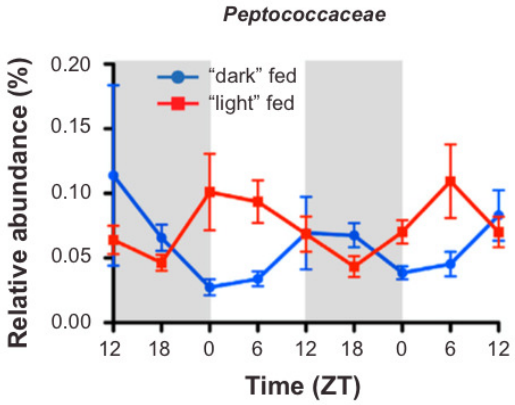
Microbiota Diurnal Oscillations Are Controlled by Feeding Time

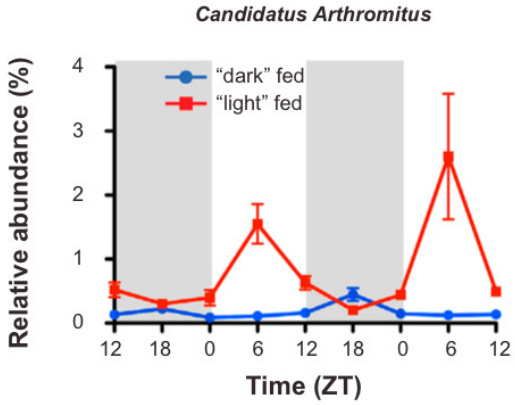
p-value

Most cycling OTUs appeared to exhibit a phase shift of about 12 hr upon modification of feeding times, suggesting direct control of micro- biota rhythms by feeding times.



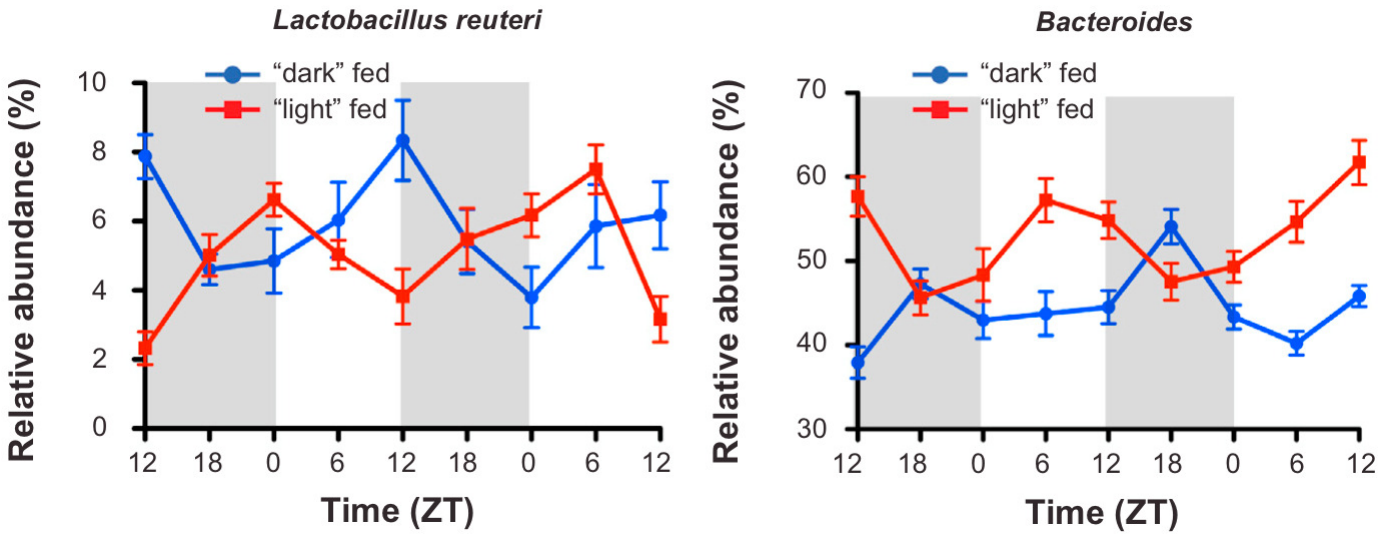




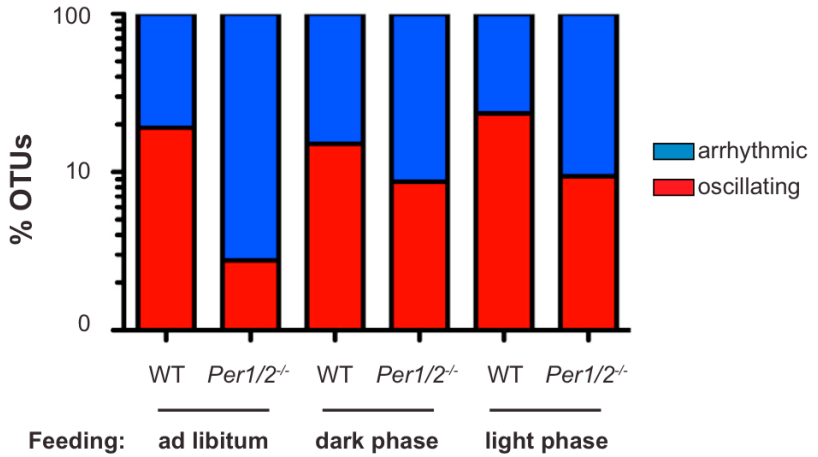


Such a phase shift was, for instance, observed in the case of *Bacteroides acidifaciens*, *Lactobacillus reuteri*, and *Peptococcaceae*. We also observed cases of de novo or enhanced rhythmicity in the light-phase-fed groups, as exemplified by *Candidatus Arthromitus* . **These results suggest that feeding times influence daily fluctuations in microbiota composition and that the oscillations in abundance of commensal bacteria can be controlled by scheduled feeding.**

Per1/2$^{-/-}$ (deficient in a functional host clock)

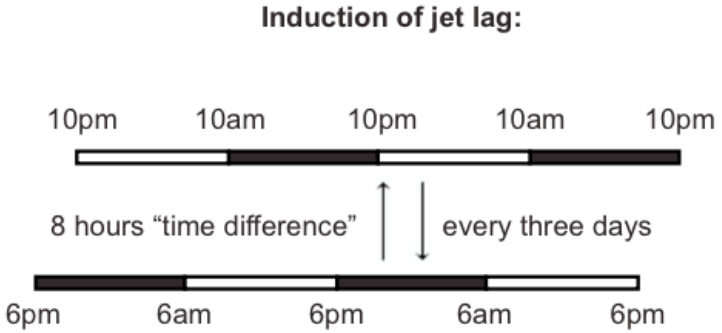


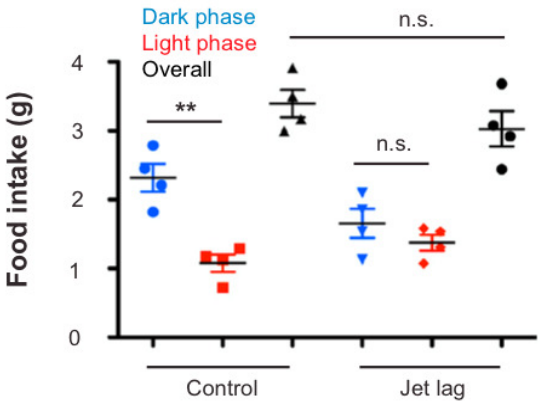
Taken together, our results show that rhythmicity of food intake dictates daily oscillations in microbiota composition and that microbiota rhythmicity is a flexible process that can be lost or regained in response to changed feeding behaviors. Thus, feeding times couple the circadian patterns of host behavior to diurnal fluctuations in microbiota composition and function.

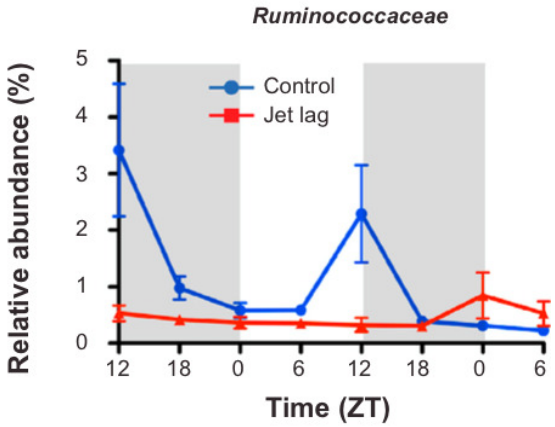


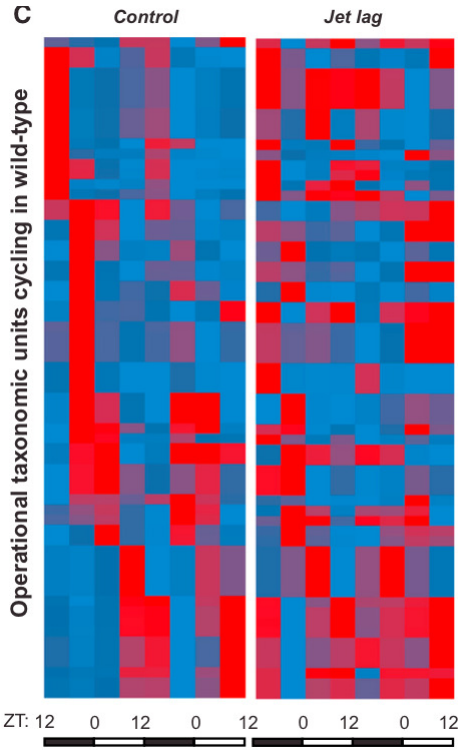
Environmental Disruption of Normal Sleep Patterns Induces Loss of Microbiota Diurnal Rhythmicity and Dysbiosis

Given our finding that rhythmic food intake induces diurnal fluctuations in the microbiota, we examined whether these disruptions of rhythmic behavior by jet lag would also impair diurnal oscillations in microbiota composition.

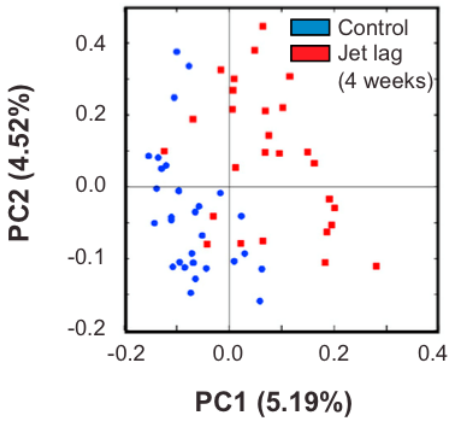




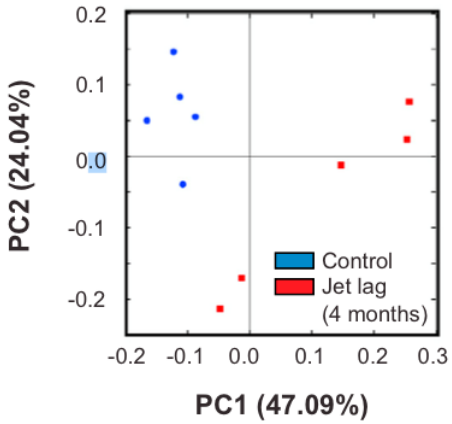


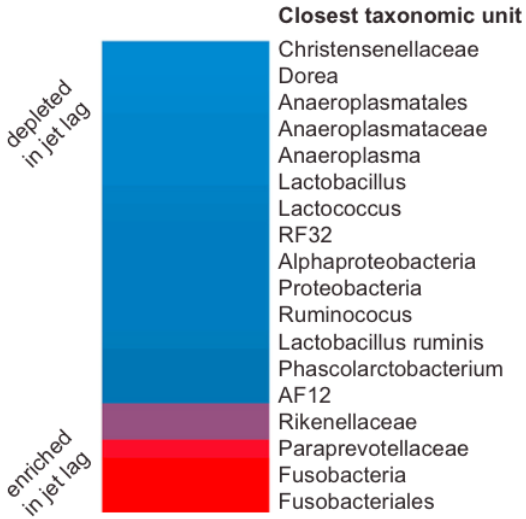


Beta diversity of gut microbial communities in control and jet lag mice after 4 weeks of time shifts. Samples are pooled from different times of the day.



Beta diversity of gut microbial communities in control and jet lag mice after 4 months of time shifts.

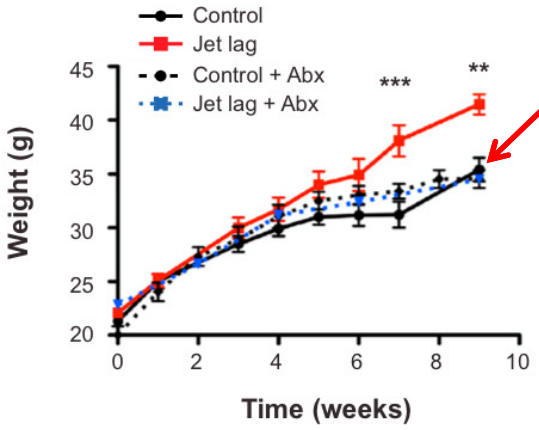


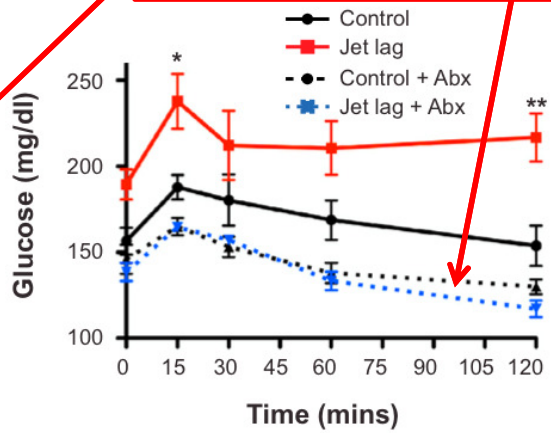


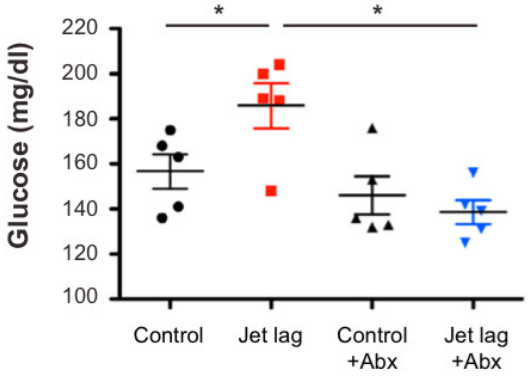
Heatmap representation of changes in microbial composition induced by jet lag.

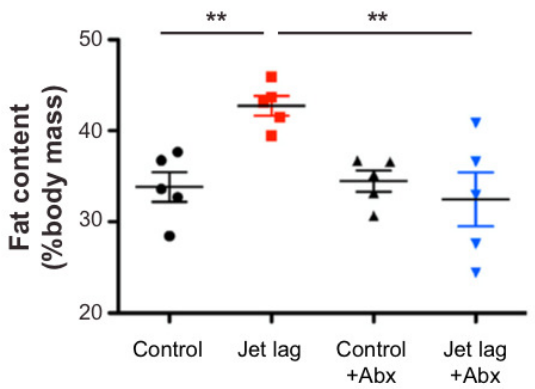
We fed jet-lagged and control mice a high-fat diet, containing 60% of caloric energy from fat, thereby mimicking human dietary habits predisposing to the metabolic syndrome. Indeed, as early as 6 weeks after instating of high-fat diet, time-shifted mice exhibited enhanced weight gain and exacer- bated glucose intolerance as compared to mice maintained on normal circadian rhythmicity.

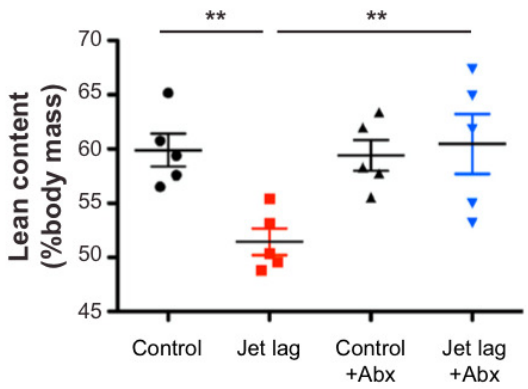
we hypothesized that alterations in microbiota composition may contribute to this metabolic pheno- type. Indeed, wide-spectrum antibiotic treatment for the dura- tion of jet lag induction (vancomycin, ampicillin, kanamycin, and metronidazole;

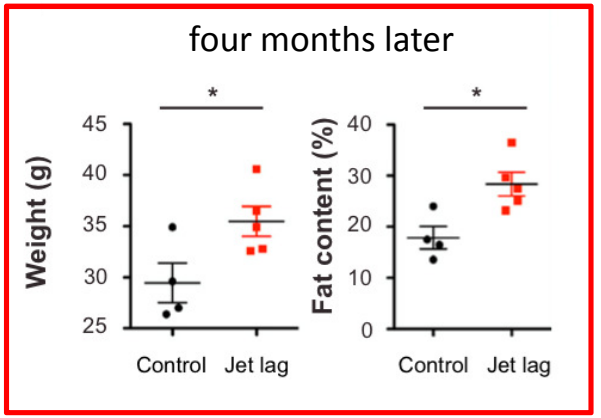


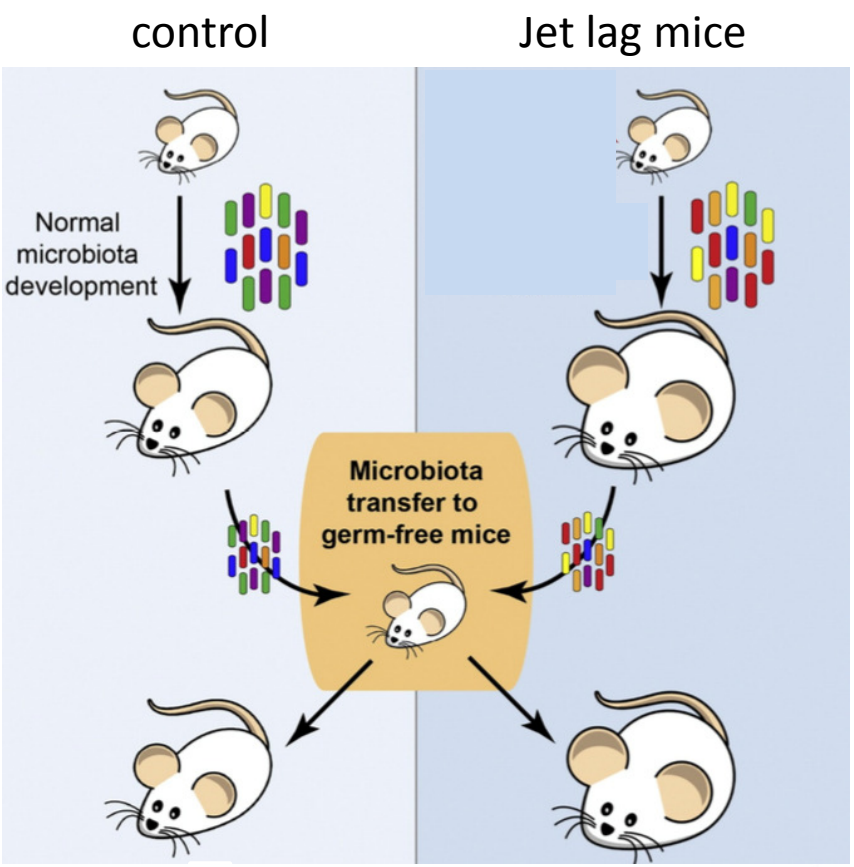


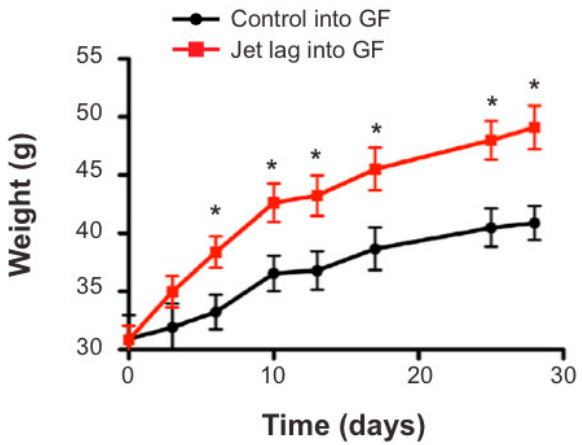


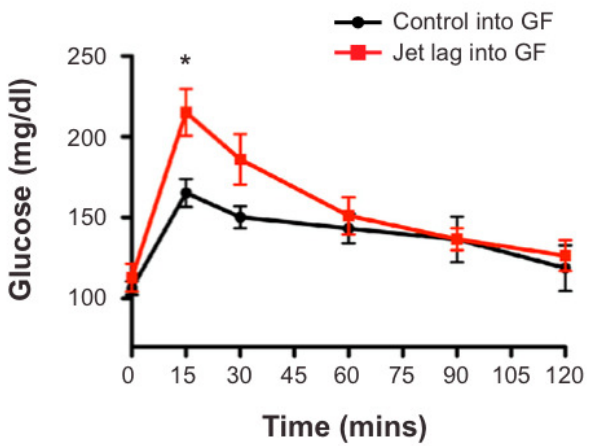


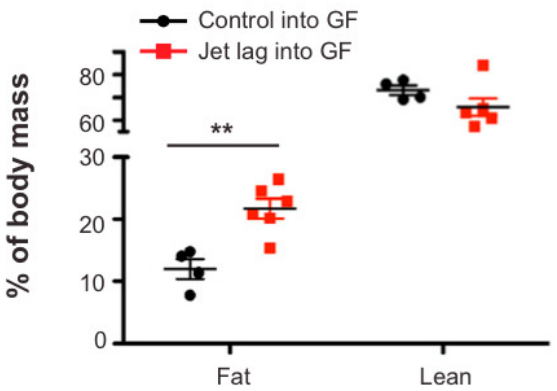




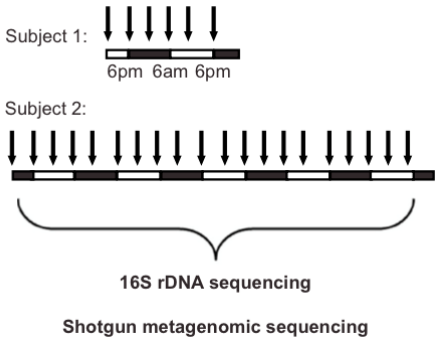

control
Jet lag mice
Normal microbiota development
Microbiota transfer to germ-free mice

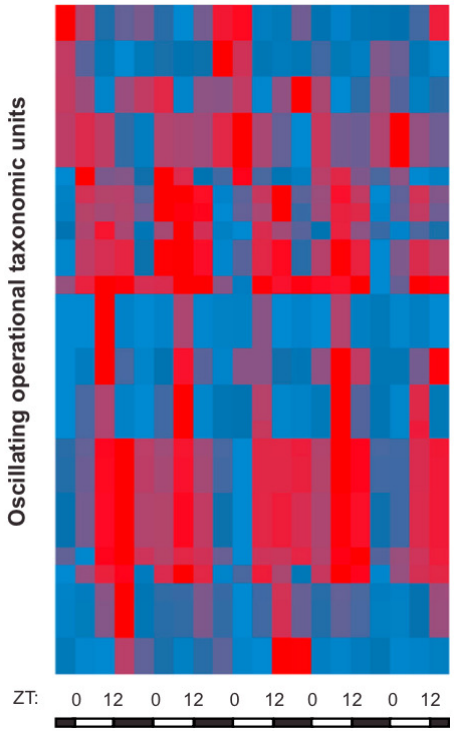

Control into GF
Jet lag into GF
Weight (g)
55
50
45
40
35
30
0
10
20
30
Time (days)

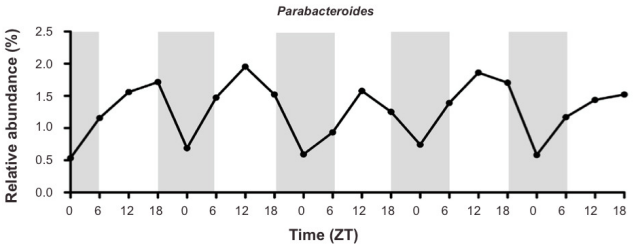

Control into GF
Jet lag into GF
Glucose (mg/dl)
250
200
150
100
0
15
30
45
60
75
90
105
120
Time (mins)

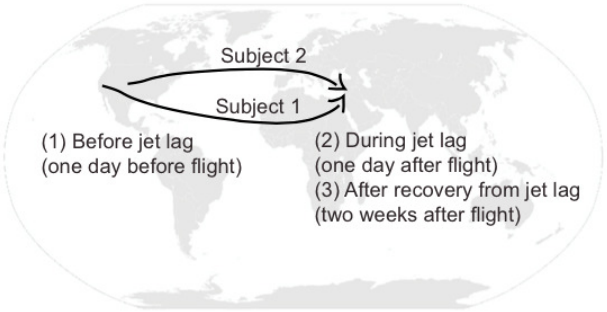

Control into GF
Jet lag into GF
% of body mass
80
60
30
20
10
0
**
Fat
Lean

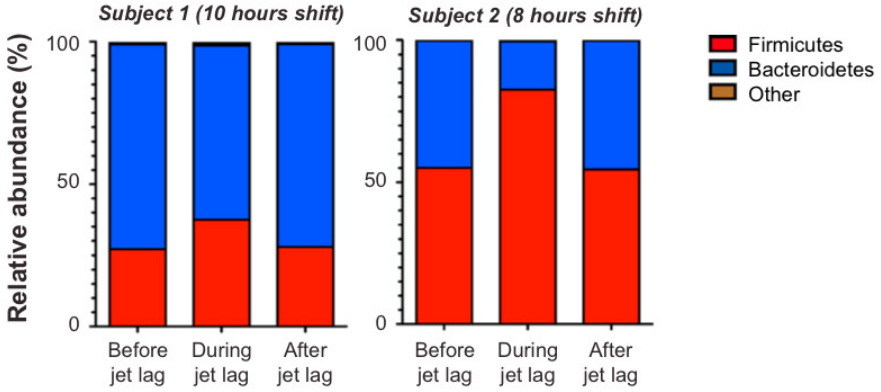
What about in humans …?

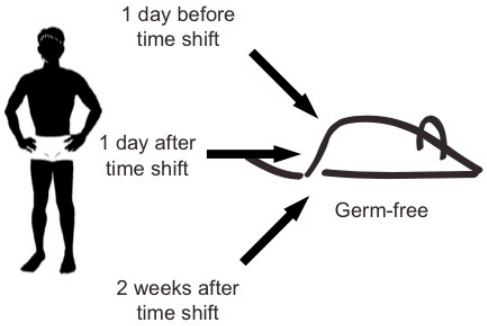


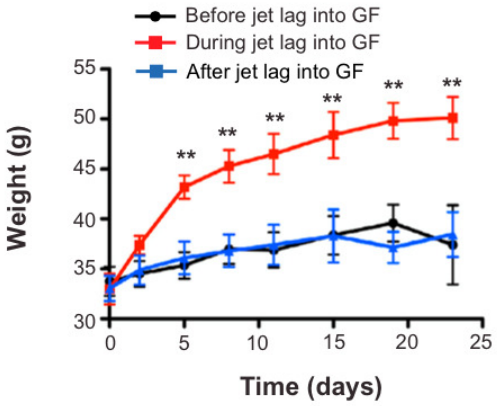


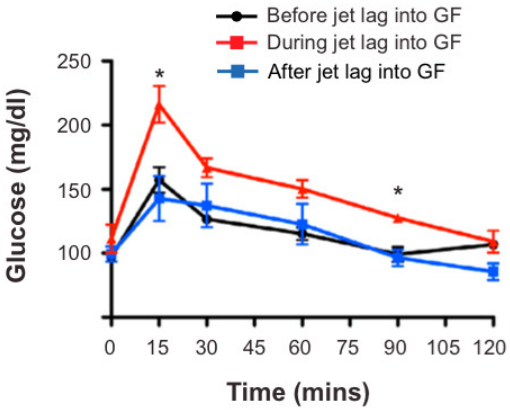



Subject 2
Subject 1
(1) Before jet lag
(one day before flight)
(2) During jet lag
(one day after flight)
(3) After recovery from jet lag
(two weeks after flight)


Subject 1 (10 hours shift)
Subject 2 (8 hours shift)
Relative abundance (%)
100
50
0
Before jet lag
During jet lag
After jet lag
Firmicutes
Bacteroidetes
Other


1 day before time shift
1 day after time shift
2 weeks after time shift
Germ-free


Before jet lag into GF
During jet lag into GF
After jet lag into GF
Weight (g)
55
50
45
40
35
30
0
5
10
15
20
25
Time (days)
**


Before jet lag into GF
During jet lag into GF
After jet lag into GF
Glucose (mg/dl)
250
200
150
100
0
15
30
45
60
75
90
105
120
Time (mins)
*