Chronodisruption by chronic jetlag impacts metabolic and gastrointestinal homeostasis in male mice

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Short title:

Chronic jetlag disrupts gut homeostasis

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ABSTRACT

Aim Chronodisruption desynchronizes peripheral clocks and leads to metabolic diseases. Feeding cues are important synchronizers of peripheral clocks and influence rhythmic oscillations in intestinal microbiota and their metabolites. We investigated whether chronic jetlag, mimicking frequent time zone traveling, affected the diurnal fluctuations in faecal short-chain fatty acid (SCFA) levels, that feed back to the gut clock to regulate rhythmicity in gut function.

Methods Rhythms in faecal SCFAs levels and in the expression of clock genes and epithelial markers were measured in the colonic mucosa of control and jetlagged mice. The entraining effects of SCFAs on the rhythm in clock gene mRNA expression was studied in primary colonic crypts. The role of the circadian clock in epithelial marker expression was studied in *Arntl*$^{-/-}$ mice.

Results Chronic jetlag increased body weight gain and abolished the day/night food intake pattern which resulted in a phase-delay in the rhythm of faecal SCFAs, that paralleled the shift in the expression of mucosal clock genes. This effect was mimicked by stimulation of primary colonic crypts from control mice with SCFAs. Jetlag abolished the rhythm in *Tnfα*, *proglucagon* and *ghrelin* expression but not in the expression of tight junction markers. Only a dampening in plasma GLP-1 but not in ghrelin levels was observed. Rhythms in *ghrelin* but not *proglucagon* mRNA expression were abolished in *Arntl*$^{-/-}$ mice.

Conclusion The altered food intake pattern during chronodisruption corresponds with the changes in rhythmicity of SCFA levels that entrain clock genes to affect rhythms in mRNA expression of gut epithelial markers.

Key words Arntl Knock Out, Circadian Clock, Colonic Crypts, Gastrointestinal hormones, Jetlag, Short-chain fatty acids

INTRODUCTION

The circadian system enables organisms to optimally adapt their physiology and behaviour to the natural light/dark rhythm.[1] The circadian system is hierarchically organized with a master clock, located in the suprachiasmatic nuclei (SCN) in the hypothalamus, regulating the clocks that are present in most other central and peripheral tissues.[2]

At the molecular level, circadian rhythms are generated through a network of positive and negative transcriptional-translational feedback loops (TTFLs) that regulate gene expression. The core clock units Circadian Locomotor Output Cycles Kaput (CLOCK) and Aryl hydrocarbon Receptor Nuclear Translocator-Like (ARNTL) form a heterodimer that controls transcription by binding to E-boxes in the promotor region of numerous downstream genes, such as *Period (Per1, Per2 and Per3) and Cryptochrome (Cry1, Cry2)*. These can in turn repress the transactivatory function of the CLOCK:ARNTL heterodimer. Another auxiliary feedback loop consists of the CLOCK:ARNTL heterodimer inducing transcription of *Rorα* and *Reverbα* that in turn can activate or repress *Arntl* transcription.[3] Besides regulating their own expression, the circadian clock in turn controls the expression of several other genes, the so-called clock-controlled genes (CCG).[4] Animals with mutations or ablations of these core clock genes show disrupted food intake patterns, body weight and metabolism.[5]

Disruption of the circadian clock system, also called chronodisruption, occurs when there is a mismatch between the intrinsic circadian clock and behaviour (activity/rest, feeding/fasting), as, for example, in shift work, social and chronic jetlag.[6] Chronodisruption has already been extensively linked to several diseases like metabolic syndrome, obesity, stroke, breast and prostate cancer. Indeed, studies in both humans and mouse models showed that chronodisruption was associated with body weight gain, altered food intake patterns, a loss in rhythmic physical activity, a dampening of the rhythmicity in the respiratory exchange ratio, glucose intolerance, dyslipidemia, gastrointestinal symptoms and diseases, but also an increased intestinal permeability that can lead to a higher susceptibility to intestinal inflammation.[7-14] In addition, chronodisruption has been shown in mice and humans to affect

the peripheral clocks in the liver and peripheral blood mononuclear cells, respectively.[6,15-18] Timing of food intake is an important synchronization cue or zeitgeber (ZT) for peripheral clocks and restricting food intake to the inactive phase in mice, can uncouple the peripheral clocks in the liver from the master clock in SCN.[19-21]

The intestinal microbiota show diurnal fluctuations that can be influenced by the host's circadian clock, as these fluctuations are lost in *Arntl*$^{-/-}$ mice, and by changes in the diet or feeding pattern. [22-25] Microbial metabolites such as short-chain fatty acids (SCFA), show diurnal fluctuations that are lost in *Arntl*$^{-/-}$ mice, blunted in diet-induced obese mice and restored by night-time restricted feeding.[25,26] Diurnal fluctuations in SCFA levels are crucial to orchestrate and maintain proper oscillations of clock genes in peripheral tissue.[25,27] For example, oral gavage of SCFAs shifts the circadian clock in the liver and kidney of mice. [25,27]

Enteroendocrine cells and their endogenous clocks in the gut are among the first cells encountering shifts in the rhythmicity of SCFAs due to chronodisruption. P/D1 cells, containing the hunger hormone ghrelin, express clock genes and timed stimulation with food-related stimuli (peptone to simulate the fed state and L-epinephrine to simulate the fasted state) induce a circadian rhythm in ghrelin release in murine ghrelinoma cells.[28,29] Rhythmic fluctuations in plasma ghrelin levels are blunted in obese people and in night shift workers.[30-32] Furthermore, the rhythmic effects of SCFA on the release of ghrelin in the colon are abolished in *Arntl*$^{-/-}$ mice.[26] The secretion of glucagon-like peptide-1 (GLP-1), a satiety hormone that stimulates insulin release, is diurnal and is blunted in *Arntl*$^{-/-}$ mice and in obese and short term sleep-deprived people with nocturnal light exposure.[33-36]

Also other cells in the gut mucosa such as immune cells, more specifically leukocytes, macrophages and T helper 17 cells that help mediate mucosal immunity, and tight junction proteins show a circadian rhythm in their protein expression levels.[10,37-43]

We hypothesize that chronodisruption induced by chronic jetlag in mice might induce alterations in dietary food intake pattern that will induce shifts in the production of microbial metabolites that can entrain peripheral clocks in the gut epithelium to affect the rhythmic expression of gut epithelial cell markers that regulate gut function. To investigate the entraining

capabilities of SCFAs on circadian clock mRNA expression, SCFAs will be administered to synchronized primary colonic crypts from control mice. Using a *Arntl*$^{-/-}$ mice model we will investigate the role of the circadian clock in the mRNA expression of several gut metabolic markers.

RESULTS

Jetlag promotes body weight gain and changes in food intake pattern

Jetlagged mice gained more weight compared to control mice (time x condition: $P < 0.01$) (Figure 1a) during the 4 weeks of jetlag induction without changing their total daily caloric intake (Figure 1b). Nevertheless, the jetlagged mice had an altered day/night food intake pattern compared to the control mice (time x condition: $P < 0.001$) (Figure 1c). While control mice ate most of their calories during the night ($P < 0.001$), there was no significant difference between the amount of calories eaten during night or day in jetlagged mice.

Jetlag induces shifts in faecal SCFA concentrations

To investigate the possible effect of the changed food intake pattern on the production of microbial metabolites, SCFA concentrations were measured in the luminal content of the distal colon over 24 hours. Faecal concentrations of acetate showed no diurnal rhythm in both control and jetlagged mice (Figure 2a), while propionate ($P_{Cosinor} < 0.05$) and butyrate ($P_{Cosinor} < 0.01$) concentrations showed a diurnal rhythm in the control mice with peak concentrations at *zeitgeber time* (ZT) 4:47 and ZT 3:04, respectively (Figure 2b-c). Jetlag delayed the acrophase of the rhythmic fluctuations of propionate and butyrate by 5:19 ($P < 0.01$) and 2:43 ($P < 0.05$), respectively. Jetlag did not affect the total faecal SCFA concentration over 24h between control (23.2 mM) and jetlagged (25.5 mM) mice. The cosinor parameters for all experiments performed in control and jetlagged mice are summarized in Table 1.

Shifts in circadian clock gene mRNA expression in the colon and stomach mimic the shifts in SCFA concentrations

SCFAs are known to affect circadian clock expression in peripheral tissues. Therefore, clock gene expression was studied in the mucosa of the distal colon and stomach of control and jetlagged mice.

The mRNA expression of the positive regulators of the circadian clock *Arntl* ($P_{Cosinor} < 0.001$) and *Clock* ($P_{Cosinor} < 0.001$) and the negative regulator *Reverbα* ($P_{Cosinor} < 0.001$) in the mucosa of the distal colon showed diurnal rhythmicity in control mice and peaked at ZT 2:19, ZT 2:43 and ZT 10:37, respectively. The acrophases of *Arntl*, *Clock* and *Reverbα* were delayed by 4:20 ($P < 0.001$), 2:10 ($P < 0.05$) and 4:53 ($P < 0.001$) in jetlagged mice (Figure 3a-c). In addition, jetlag also decreased ($P < 0.01$) the amplitude of the rhythm of *Reverbα* expression by 57% in the distal colonic mucosa (Figure 3c). Furthermore, *Arntl* and *Clock* mRNA expression peaked together in control mice ($P = 0.45$), as they form a heterodimer to exert their function, but in jetlagged mice *Arntl* and *Clock* mRNA expression no longer peaked together ($P < 0.01$). Expression patterns of the negative regulator *Per2* were also measured in the distal colonic mucosa and showed similar results as *Reverbα* expression (Figure S1). Interestingly, the respective shifts between control and jetlagged mice in neither faecal propionate nor butyrate levels differed significantly from the respective shifts observed for *Arntl*, *Clock*, *Reverbα* and *Per2* mRNA expression. Moreover, both *Arntl* and *Clock* mRNA expression peaked together with the faecal propionate and butyrate concentration in both control and jetlagged mice which in turn probably induced the shift in the mRNA expression of the other clock genes *Reverbα* and *Per2*. (Figure 3d).

Similar results were found in the mucosa of the stomach where the mRNA expression of *Arntl* ($P_{Cosinor} < 0.001$), *Clock* ($P_{Cosinor} < 0.001$) and *Reverbα* ($P_{Cosinor} < 0.001$) showed diurnal fluctuations in control mice and peaked at ZT 0:37, ZT 22:22 and ZT 9:37, respectively. The acrophases of *Arntl* and *Reverbα* were delayed by 4:44 ($P < 0.001$) and 4:36 ($P < 0.001$), while the rhythm in *Clock* mRNA expression was lost in jetlagged mice as shown in Table 1 and in Figure 4 which summarizes the acrophases of all measured parameters measured in control and jetlagged mice.

Interestingly, the acrophase of *Arntl*, *Clock* and *Reverbα* mRNA expression peaked earlier in the stomach mucosa than in the distal colonic mucosa in control mice. A significant time delay in the colon was observed compared to the stomach of 1:41 ($P < 0.001$) for *Arntl,* 4:20 ($P < 0.001$) for *Clock* and 1:00 ($P < 0.001$) for *Reverbα* (Figure 3e). In jetlagged mice, similar shifts

in acrophases were found for *Arntl* (0:58) ($P < 0.05$) and *Reverbα* (1:16) ($P < 0.001$). No rhythm was observed in *Clock* mRNA expression in the stomach of jetlagged mice.

SCFAs induce a shift in clock gene expression in primary colonic crypts *in vitro*

The changes in faecal SCFAs may be a potential timing cue that affects circadian clock gene transcription. The hypothesis that the observed phase-delay in faecal SCFAs provokes the shift in the circadian clock gene expression was investigated in synchronized primary colonic crypts that do not receive input from the master clock in the brain. To investigate this hypothesis primary colonic crypts of control mice were synchronized with dexamethasone and incubated with either Dulbecco's Modified Eagle Medium (DMEM) (Control) or a mixture of SCFAs (24 mM). Representative pictures of the primary colonic crypts at time of isolation and after synchronization are shown in Figure S3 (a-b). *Arntl*, *Reverbα* and *Per2* mRNA expression showed diurnal rhythmicity in control and SCFA-treated crypts (Figure 5a-c). Stimulation of primary colonic crypts with a mixture of SCFAs that mimics the measured faecal levels in mice resulted in a phase delay of 2:38 ($P < 0.05$) in *Arntl* mRNA expression, 4:46 in *Reverbα* ($P < 0.001$) mRNA expression and 4:36 ($P < 0.01$) in *Per2* mRNA expression.

Jetlag affects the rhythmicity of gastrointestinal hormones

In the distal colonic mucosa, *Proglucagon* mRNA expression, the precursor for GLP-1, was diurnal ($P_{Cosinor} < 0.001$) in control mice, peaking at ZT 5:07, while rhythmicity was lost in jetlagged mice (Figure 6a). In addition, the acrophase of *Proglucagon* mRNA expression (ZT 5:07) did not differ statistically from the acrophase of the faecal propionate and butyrate concentrations and from the acrophase of the mRNA expression of the SCFA receptor, *Ffar2* (ZT 4:06) in the distal colonic mucosa of control mice (Figure 4). Furthermore, the observed rhythmicity in *Ffar2* mRNA expression in control mice ($P_{Cosinor} < 0.05$) was lost in jetlagged mice (Figure 6b). Plasma GLP-1 concentrations showed diurnal fluctuations in both control and jetlagged mice ($P_{Cosinor} < 0.01$) but the amplitude was dampened ($P < 0.05$) by 60% in the

jetlagged mice (Figure 6c). Blood glucose levels fluctuated ($P_{Cosinor}$ < 0.01) and peaked at ZT 6:16 in control mice while there was only a trend ($P_{Cosinor}$ = 0.06) towards rhythmicity in jetlagged mice (Figure 6d).

In the mucosa of the stomach, the major production site of ghrelin, *Ghrelin* mRNA expression fluctuated diurnally ($P_{Cosinor}$ < 0.05) in control mice and was peaking at ZT 5:07, while this rhythm was lost in jetlagged mice (Figure 7a). The effect of jetlag on ghrelin O-acyltransferase (*Goat*) expression, the enzyme that catalyses the octanoylation of ghrelin necessary for its biological activity, was measured as well.[44,45] *Goat* mRNA expression showed a diurnal rhythm ($P_{Cosinor}$ <0.05) in control (ZT 9:29) and jetlagged (ZT10:33) mice that did not differ significantly (Figure 7b) (Figure 4). Further, octanoyl, plasma ghrelin levels also remained rhythmic ($P_{Cosinor}$ < 0.05) and peaked at ZT 14 with the same amplitude in both groups (Figure 7c).

The circadian clock regulates the mRNA expression of *Ghrelin* and *Ffar2* in the distal colon

Next, we investigated whether the effect of chronic jetlag on changes in the diurnal fluctuations of the genes of interest is regulated by the circadian clock and is not due to direct effects of for instance the shifts in faecal SCFAs via histone deacetylase inhibition. To investigate the role of the circadian clock in the rhythmic mRNA expression of *Ghrelin*, *Proglucagon* and *Ffar2* in the distal colonic mucosa, mRNA expression was investigated in *Arntl*$^{-/-}$ and their wild type (WT) littermates at ZT 4 and ZT 16. *Ghrelin* mRNA expression was significantly higher at ZT 4 than at ZT 16 in the WT littermates (P < 0.05), while no difference was observed between the two ZTs in *Arntl*$^{-/-}$ mice (Figure 8a). Similarly, *Ffar2* mRNA expression was significantly lower at ZT 16 compared to ZT 4 (P < 0.05) in WT but not in *Arntl*$^{-/-}$ mice, although an upregulation (P < 0.001) of *Ffar2* mRNA expression was observed over both ZT's (Figure 8b). *Proglucagon* mRNA expression did not differ between ZT 4 and 16 in both genotypes (Figure 8c).

Jetlag affects the rhythmicity of *Tnfα* expression but not of tight junction markers

The mRNA expression of inflammatory marker *Tumor necrosis factor α* (*Tnfα*) showed diurnal rhythmicity in the distal colonic mucosa in control mice ($P_{Cosinor} < 0.01$), peaking at ZT 8:31, while rhythmicity was lost in jetlagged mice (Figure 9a). *Interleukin 1β* (*Il1β*) was not expressed in the distal colonic mucosa of both groups. *Ocln* mRNA expression was diurnal in both groups ($P_{Cosinor} < 0.05$), peaking at ZT 4 but was not affected by jetlag. No rhythm in *Cldn1* and *Tjp1* mRNA expression was observed in neither control nor jetlagged mice (Figure 9b-c) (Table 1).

DISCUSSION

In the present study, we showed that chronic jetlag enhances body weight gain and alters the food intake pattern without an increase in consumed calories. The alteration of the day/night food intake pattern corresponds with a phase delay in the faecal SCFA levels that was paralleled by a similar phase delay in clock gene expression in the mucosa of the stomach and colon. The shift in clock gene expression was mimicked by stimulation of primary colonic crypts with SCFAs. Although jetlag abolished the rhythm in *proglucagon* and *ghrelin* mRNA expression, only a dampening in plasma GLP-1 levels was observed and no change in plasma ghrelin levels suggesting that it is unlikely that they change the food intake pattern. Studies in *Arntl*$^{-/-}$ mice showed that *Ghrelin* and *Ffar2* but not *Proglucagon* mRNA expression is regulated by the circadian clock. The rhythm in *Tnfα* mRNA expression in the distal colonic mucosa was abolished by jetlag, but not the tight junction markers of which only *Occludin* was rhythmic. In conclusion, altered feeding cues affecting the rhythmicity of microbial metabolites during chronic jetlag shift the gut clock that regulates rhythmic mRNA expression of many epithelial markers that contribute to gut homeostasis.

Our results show an increased body weight and an altered food intake pattern without an increase in daily calorie consumption in the jetlagged mice. Comparable findings were reported in a similar model of jetlag and in other models of chronodisruption, induced by exposing mice to dim light at night or to a high-fat diet.[22,46-49] Food intake is known to be strongly regulated by the arcuate nucleus (ARC) in the hypothalamus that produces AgRP, which is also influenced by the circadian clock.[50-53] Cedernaes et al. showed that both mice with a forebrain-specific ablation of *Arntl* (where the hypothalamus and SCN is located) and mice with an AgRP-specific ablation of *Arntl* exhibited a significant reduction in food intake during the dark period and an increased intake during the light period.[52] The changed food intake pattern observed in the jetlagged mice can possibly be explained by a central (light/dark cycle-driven) disruption of the circadian clock in the arcuate nucleus that centrally controls feeding rhythms. However, this was not addressed in the current study. Nonetheless, altered food intake patterns will have an

important influence on the function of the gastrointestinal tract that acts as a peripheral system involved in the short-term regulation of food intake. Meal-related fluctuations in gut hormones feedback to the arcuate nucleus to regulate hunger and satiety.[54-57]

Food is mainly digested and absorbed in the small intestine, but non-digestible dietary fibres are fermented by the microbiota to SCFAs in the colon. In agreement with previous findings from our group, we confirmed that faecal propionate and butyrate levels show diurnal fluctuations that peak in the morning in control mice.[58] The altered day/night food intake pattern in jetlagged mice concurred with a phase delay in the propionate and butyrate peak. Restoring the food intake pattern to the active phase could possibly avoid the phase delay in faecal SCFAs. A previous study already confirmed that restoration of the disturbed food intake pattern by night-time restricted feeding in chronodisrupted *Arntl* $^{-/-}$ mice restored diurnal fluctuations in caecal SCFA concentrations and the rhythmic effect of SCFAs on octanoyl ghrelin release in colonic explants. In addition, the expression of other clock genes (like *Clock*) was enhanced in the colonic mucosa.[26]

Our data confirm the presence of a circadian clock system in the distal colonic and gastric mucosa. The observed phase shift in clock gene expression in the distal colonic mucosa due to chronic jetlag is probably caused by the time-shift in the peak concentration of the diurnal faecal SCFA levels. This is implied by our results observed in the primary crypt model where SCFAs mimicking faecal concentrations shifted the circadian clock gene expression similarly to the phase delay observed in jetlagged mice. Indeed, SCFAs have previously been shown to be important entraining signals for peripheral clock genes. Oral gavage of SCFAs induced circadian clock phase shifts in mouse liver, kidney and submandibular gland.[27] In hepatic organoids addition of butyrate shifted the rhythmicity and amplitude of the clock genes *Per2* and *Arntl*.[25] Further, also other microbial metabolites like secondary bile acids have been shown both *in vitro* and *in vivo* to entrain peripheral circadian clocks.[59] Similarly, chronodisruption using sleep disruption abolished diurnal fluctuations in serum bile acids in mice.[60-62] Besides microbial metabolites, also metabolism can affect the circadian clock.

Hormones like insulin, leptin, ghrelin and glucagon have been demonstrated to acutely affect circadian clock expression.[63-66]

Taken together these findings suggest that luminal SCFAs are an entraining signal for clock genes in the colon. In the stomach, it is more likely that the shift in clock gene expression is caused by plasma SCFAs. Segers et al. showed that total plasma SCFA levels peaked at ZT 21 which approximates the rhythm of *Arntl* (ZT 0:37) and *Clock* (ZT 22:22) mRNA expression in the stomach of control mice. Moreover, in the same study the presence of SCFAs in the luminal content of the stomach was demonstrated, possibly originating from the chow or from coprophagic behavior.[26] This might directly influence clock gene expression in the stomach.

We next tried to elucidate whether chronic jetlag specifically affected clock gene expression in enteroendocrine cells of the gut mucosa by investigating the effect on the rhythm of mRNA expression of gut hormones. In control mice, *Proglucagon* mRNA expression showed diurnal fluctuations that peaked together with the faecal SCFA concentrations, the mRNA expression of the SCFA receptor *Ffar2* regulating GLP-1 secretion and the mRNA expression of *Arntl* and *Clock* in the distal colonic mucosa (see Figure 4). However, in jetlagged mice 24h rhythmicity of *Proglucagon* and *Ffar2* mRNA expression in the distal colonic mucosa was abolished. Similar observations were made for the effect of chronic jetlag on *Ghrelin* mRNA expression in the mucosa of the stomach. Several studies demonstrated the involvement of the circadian clock in the mRNA expression of both *Proglucagon* and *Ghrelin* in GLP-1 producing L-cells and in ghrelin producing X/A like cells, respectively.[28,29,67] Our studies in the distal colonic mucosa of *Arntl*$^{-/-}$ mice confirm that the circadian clock regulates the rhythmicity of *Ffar2* and *Ghrelin* mRNA expression. Since the expression of the core clock genes *Arntl* and *Clock* do not peak together anymore in the gut mucosa of jetlagged mice, it is likely that the formation of the CLOCK:ARNTL heterodimer that controls transcriptional activity of several clock controlled genes, like *Proglucagon* and *Ghrelin*, was affected. This may account for the loss in their rhythmic mRNA expression in the jetlagged mice.

In contrast, plasma GLP-1 levels were decreased in the jetlagged mice, while plasma octanoyl ghrelin levels were not affected. A similar dampening of the basal plasma GLP-1 levels and the GLP-1 response to an oral glucose tolerance test was reported in a human model and a rat model of chronodisruption induced by exposing humans or rats to constant light-exposure, respectively.[33,34] Changes in plasma levels of GLP-1 and ghrelin are not only affected by changes in mRNA levels in L-cells and in X/A like cells that are controlled by clock genes.[28,29,67] Their dynamics are also regulated by the rhythmicity in other hormones like leptin, insulin and glucagon that regulate energy homeostasis and that are affected during chronodisruption. For example, Kettner et al. demonstrated the presence of a circadian clock in adipose tissue, regulating leptin secretion that was disrupted by chronic jetlag in mice.[13] Plasma leptin levels that stimulate GLP-1 secretion are dampened in human models of circadian misalignment and in shift workers.[68-71] Further, also pancreatic islets express circadian clock genes that orchestrate temporal profiles of insulin and glucagon secretion which are reduced during chronodisruption and feedback to other hormones.[72] A recent study using antibiotic-induced microbial depleted and germ-free mice indicated that diurnal GLP-1 release is dependent on the intestinal microbiome and thereby support our findings for a role of SCFAs in the regulation of the metabolic clock.[73] In addition, Biancolin et al. showed that the SNARE regulatory protein secretagogin is under circadian control and is necessary for the circadian secretion of GLP-1.[36] The situation for ghrelin is even more complex since the mRNA expression of *Goat*, the enzyme that controls the posttranslational octanoylation of ghrelin was not affected by chronic jetlag. This may override the loss in rhythmicity in *Ghrelin* mRNA expression and may explain why plasma octanoyl ghrelin levels were not affected by chronic jetlag.

We conclude that the change in food intake pattern in jetlagged mice is not induced by the changes in the rhythm of plasma levels of the satiety hormone GLP-1 nor is it triggered by alterations in the rhythm of plasma levels of the hunger hormone ghrelin.[74-76] Rather, we hypothesize that the dampening in plasma GLP-1 levels is caused by the change in the food intake pattern in jetlagged mice. Gonnissen et al. investigated the effect of a phase advance

or phase delay of the 24-h cycle in humans and showed that meal-related blood variables such as GLP-1 and ghrelin followed the new meal patterns. This is in contrast to our findings in plasma ghrelin levels but is in agreement with the changes in plasma GLP-1 which followed the changes in food intake rhythmicity.[77]

ARNTL plays an important role in the diurnal fluctuations of immune cells and cytokines in the small intestine and stomach.[78,79] This is in agreement with previous findings in a genetic model of chronodisrupton which showed that diurnal fluctuations in *Tnfα* mRNA expression in the small intestine were abolished in *Arntl*$^{-/-}$ mice and *Arntl*$^{-/-}$ organoids. It was concluded that the rhythmic expression of *Tnfα* is an important driver of rhythmic epithelial proliferation in epithelial precursors to replace damaged epithelial cells during a pathological state.[78]

Studies inducing chronodisruption by constant light exposure or by exposing mice to weekly circadian shifts showed an increase in permeability and a higher susceptibility to inflammation.[43,80,81] Oh-oka et al. showed 24h rhythmicity in *Ocln* and *Cldn1* mRNA expression in the large intestine that was under control of the clock components ARNTL and CLOCK.[10] This is not in accordance with our results, we observed a circadian rhythm in *Ocln*, but not in *Cldn1* and *Tjp1* mRNA expression in the distal colonic mucosa in control mice. Furthermore, there was no difference between control and jetlagged mice in the expression of these tight junction markers. In conclusion, although permeability itself nor the protein levels were measured in our study, we speculate that chronic jetlag does not affect gut permeability but can possibly affect epithelial regeneration during a pathological state.

It is important to note that our model of chronic jetlag mimics frequent time zone travelling in which the subjects are exposed to three days of jetlag for four consecutive weeks. To really mimic rotating shift work, the mice should be forced to be active during the inactive phase by for example using a running wheel, because chronodisrupted mice lose the rhythm in their locomotor activity similar to the change in the food intake pattern.[22,47] Another limitation of the study is that some of the findings are based on statistically proven correlations but therefore do not imply causation, this needs to be addressed in more detail in future studies.

In conclusion, we showed that the altered food intake pattern due to chronic jetlag paralleled the phase delay in the faecal SCFAs peak. This phase delay affects the acrophase of gut clock genes, as mimicked *in vitro* in primary colonic crypts, and may disrupt the formation of the CLOCK:ARNTL heterodimer, and thus the transactivatory function, that regulates the mRNA expression of several genes including *Ghrelin* and *Ffar2* that regulate gut homeostasis. Future studies are warranted to show whether restoring the food intake pattern to the active phase could avoid the phase delay in faecal SCFAs, and hence the disruption of the circadian clock and the related metabolic consequences.

MATERIAL AND METHODS

Mice studies

Mice

Wild-type C57BL/6J mice were obtained at the age of 12 weeks from Janvier Labs (Le Genest Saint Isle, France). *Arntl*$^{+/-}$ mice (gift R. Lijnen, KU Leuven, Leuven, Belgium)[82] were bred to generate WT and *Arntl*$^{-/-}$ mice in the animal facility of the KU Leuven and genotyped by PCR on total genomic DNA from the ear. Mice had *ad libitum* access to chow and water and were housed in a temperature-controlled environment. All experiments were approved by the Ethical committee for Animal Experiments of the KU Leuven and carried out in accordance with the approved guidelines.

Experimental design

Chronic jetlag model

Control mice were kept under a 12h/12h light/dark-cycle (Zeitgeber time (ZT) 0 = lights on (= 8 a.m.)). Jetlagged mice were housed for four days a week under a 12h/12h light/dark-cycle (ZT 0 = lights on) and were shifted 8 hours forward for the remaining three days of the week (ZT 8 = lights on), after which the jetlagged mice were shifted back to the normal 12h/12h light/dark-cycle (ZT 0 = lights on), for four consecutive weeks (Figure S2). Body weight was monitored once a week at the second day of the normal light/dark cycle (ZT 0 = lights on) during the four weeks of jetlag induction and at the time of euthanasia. Food intake was measured in the fourth week of jetlag induction at the second day of the normal light/dark cycle. After four weeks of jetlag induction, the mice (male, age 16-17 weeks) were euthanized over the course of 24 hours at 4-hour intervals. Jetlagged mice were euthanized one to three days after these mice were in the same light/dark cycle as control mice, and ZTs were synchronized (i.e., ZT 0 of jetlag mice corresponded to ZT 0 of control mice), to avoid the possible acute effects of the last time shift. The luminal content of the distal colon was collected for measurement of SCFA concentrations and stored at -80°C. Blood was collected via cardiac

puncture and processed for plasma ghrelin and plasma GLP-1 measurements. Blood glucose concentrations were measured using a glucometer. The mucosa was dissected from the stomach and distal colon, stored in RNAlater (Qiagen, Hilden, Germany) and processed for quantitative real-time PCR (qRT-PCR).

Arntl$^{-/-}$ model

Arntl$^{-/-}$ and their WT littermates (male, age 12-15 weeks) were euthanized at ZT 4 and 16. The mucosa was dissected from the distal colon, stored in RNAlater (Qiagen, Hilden, Germany) and processed for quantitative real-time PCR (qRT-PCR).

Primary culture study

Wild-type C57BL/6J mice (male, age 14 weeks) were euthanized and the colon was removed. The colonic mucosa was dissected free of smooth muscle layer, minced, rinsed, and digested several times with collagenase XI (0.35 mg ml-1) in DMEM at 37°C. Resulting cell suspensions were centrifuged and resuspended in DMEM supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin, 1% L-glutamine, and 10 µM Y-27632. Cell aliquots were seeded on Matrigel (1.4% v v-1) coated 24-well plates. Following 20h incubation at 37°C., the cells were incubated for 2h at 37°C in DMEM supplemented with 200 nM dexamethasone to synchronize the cells. After synchronization, the cells were immediately incubated with either DMEM (Control) or DMEM containing SCFAs mimicking the faecal concentration measured in the control mice of the jetlag experiment (24 mM; ratio: 17.4 mM Acetate, 3 mM Propionate, 3.6 mM Butyrate). Samples for qPCR analysis were taken every 4 hours for 36 hours. Circadian rhythmicity in mRNA expression of the clock genes was determined between ZT 16 and 36 to avoid the acute effect of dexamethasone.

Analysis of faecal SCFA concentrations

Faecal samples (100 mg) were suspended in 1 mL of saturated NaCl (36%) solution. An internal standard (50 µL 2-ethylbutyric acid) was added and the samples were homogenized using glass beads. SCFAs were extracted with ether (3 mL) in the presence of H_2SO_4 (150 µL).

The ether layer was collected and dried by Na_2SO_4 (50 mg). Analysis was done by gas chromatography-flame ionization detector (Agilent, Santa Clara, CA), with an injection volume of 0.5 µL. The resulting chromatograms were processed using the Xcalibur software (Thermo Fischer Scientific, Waltham, MA).

Quantitative real-time PCR

Total RNA was isolated from the mucosa using the RNeasy Mini Kit (Qiagen, Hilden, Germany). Total RNA from the colonic cultures was isolated using the Relia Prep Kit (Promega, Madison, WI, USA), Both total RNA preparations were treated with the Turbo DNA-free™ kit (Thermo Fisher Scientific, Waltham, MA) and reverse transcribed to cDNA using qScript cDNA SuperMix (Quanta BioSciences, Gaithersburg, MD) according to the manufacturer's instructions. qRT-PCR was performed using the Lightcycler 480 (Roche Diagnostics, Basel, Switzerland) with the Lightcycler 480 Sybr Green I Master mix (Roche Diagnostics, Basel, Switzerland). A calibrator was used to correct for inter-run variability between plates. Results from the mucosa were expressed relative to the geometric mean of the normalized expression of three stable housekeeping genes determined according to the method of Vandesompele ($2^{-\Delta\Delta Ct}$) (Mucosa stomach: cyclophilin (*Cycloph*), TATA box binding protein (*Tbp*), hydroxymethylbilane synthase (*Hmbs*); Distal colonic mucosa (chronic jetlag model): *Tbp*, *Cycloph*, *β-actin*; Distal colonic mucosa (*Arntl*$^{-/-}$ model): glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*), *Hmbs*, *Tbp*) and that did not show diurnal rhythms in expression levels.[83] Results from the colonic cultures were expressed relative to both *Tbp* expression levels that did not show diurnal rhythms in expression levels and to ZT 0 (the first moment after synchronization) that is the same in both control and SCFA treated primary colonic crypts. Primer sequences are shown in Table 2.

Plasma hormone measurements

Plasma samples were obtained from free-feeding animals. Plasma samples for ghrelin were acidified (0.1 N HCl) and supplemented with AEBSF to a final concentration of 80 mM (Sigma-

Aldrich, Saint Louis, Missouri), extracted on a Sep-Pak C18 column (Waters Corporation, Milford, MA) and vacuum-dried. The radioimmunoassay for octanoyl ghrelin was performed as previously described.[84] Plasma samples for GLP-1 were supplemented with dipeptidyl peptidase 4 inhibitor (10 µL mL-1) and GLP-1 was measured using a Mesoscale assay (K150JWC-2, Mesoscale Discovery, Rockville, Maryland) according to the manufacturer's protocol.

Statistical analysis

Results are presented as mean ± SEM if not stated otherwise. Comparison of body weight and food intake between control and jetlagged mice was performed using a linear mixed model followed by planned comparison and post-hoc testing. All statistical analyses were performed in SAS Studio University Edition 9.4. Since the qPCR data was distributed in a non-normal and/or non-homogeneous manner, log-transformed data was used for all further analyses of the qPCR data. Diurnal rhythm analysis in the jetlag model was calculated using the cosinor procedure, in which the best-fitting cosine curve for a data set was calculated.[85] Probability values for the best fitting cosine curve are indicated as $P_{cosinor}$. Differences in acrophase (the time point where the fitted cosine curve reaches its maximum), mesor and amplitude between control and jetlagged mice within one measured parameter were compared using non-linear model analysis. Differences in acrophase, mesor and amplitude between 2 different measured parameters and comparison of the shifts in acrophases between 2 measured parameters was performed using non-linear mixed model (Proc NLMixed). Two-way analysis of variance was used to compare the effect of genotype and ZT in the *Arntl*$^{-/-}$ model (Proc GLM). In the colonic crypt model diurnal rhythmicity was analysed using the cosinor procedure. To compare the acrophases between control and SCFA-treated crypts a non-linear mixed model was used using mouse as subject (Proc NLMixed). Significance was accepted at the 5% level.

ACKNOWLEDGMENTS

The authors thank Linda Nys and Greet Vandermeulen for their skilful technical assistance.

FOOTNOTES

Contributors

L.D., A.S., K.V., and I.D. conceived and designed the experiments. L.D. and T.T. performed the experiments. L.D., T.T. and I.D. analysed the results. L.D. and I.D. wrote the manuscript. All authors reviewed the manuscript.

Conflict of interest

None declared.

Funding

This work is supported by a FWO-SB grant (1S27618N). The funder of the study had no role in study design, data collection, data analysis, data interpretation or writing of the report.

Ethics approval

Ethical Committee for Animal Experimentation of the KU Leuven.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Edery I. Circadian rhythms in a nutshell. *Physiol Genomics.* 2000;3(2):59-74.
2. Reppert SM, Weaver DR. Coordination of circadian timing in mammals. *Nature.* 2002;418(6901):935-941.
3. Partch CL, Green CB, Takahashi JS. Molecular architecture of the mammalian circadian clock. *Trends Cell Biol.* 2014;24(2):90-99.
4. Mazzoccoli G, Pazienza V, Vinciguerra M. Clock genes and clock-controlled genes in the regulation of metabolic rhythms. *Chronobiol Int.* 2012;29(3):227-251.
5. Laermans J, Depoortere I. Chronobesity: role of the circadian system in the obesity epidemic. *Obes Rev.* 2016;17(2):108-125.
6. Segers A, Depoortere I. Circadian clocks in the digestive system. *Nat Rev Gastroenterol Hepatol.* 2021.
7. Bae SA, Fang MZ, Rustgi V, Zarbl H, Androulakis IP. At the Interface of Lifestyle, Behavior, and Circadian Rhythms: Metabolic Implications. *Front Nutr.* 2019;6:132.
8. Mukherji A, Kobiita A, Damara M, et al. Shifting eating to the circadian rest phase misaligns the peripheral clocks with the master SCN clock and leads to a metabolic syndrome. *Proc Natl Acad Sci U S A.* 2015;112(48):E6691-6698.
9. Knutsson A, Boggild H. Gastrointestinal disorders among shift workers. *Scand J Work Environ Health.* 2010;36(2):85-95.
10. Kyoko O-o, Kono H, Ishimaru K, et al. Expressions of tight junction proteins Occludin and Claudin-1 are under the circadian control in the mouse large intestine: implications in intestinal permeability and susceptibility to colitis. *PloS one.* 2014;9(5):e98016-e98016.
11. Wang S, Lin Y, Yuan X, Li F, Guo L, Wu B. REV-ERBalpha integrates colon clock with experimental colitis through regulation of NF-kappaB/NLRP3 axis. *Nat Commun.* 2018;9(1):4246.

12. Thaiss CA, Zeevi D, Levy M, et al. Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell.* 2014;159(3):514-529.
13. Kettner NM, Mayo SA, Hua J, Lee C, Moore DD, Fu L. Circadian Dysfunction Induces Leptin Resistance in Mice. *Cell Metab.* 2015;22(3):448-459.
14. Crowther ME, Ferguson SA, Vincent GE, Reynolds AC. Non-Pharmacological Interventions to Improve Chronic Disease Risk Factors and Sleep in Shift Workers: A Systematic Review and Meta-Analysis. *Clocks Sleep.* 2021;3(1):132-178.
15. Christie S, Vincent AD, Li H, et al. A rotating light cycle promotes weight gain and hepatic lipid storage in mice. *Am J Physiol Gastrointest Liver Physiol.* 2018;315(6):G932-g942.
16. Barclay JL, Husse J, Bode B, et al. Circadian desynchrony promotes metabolic disruption in a mouse model of shiftwork. *PLoS One.* 2012;7(5):e37150.
17. Cuesta M, Boudreau P, Cermakian N, Boivin DB. Rapid resetting of human peripheral clocks by phototherapy during simulated night shift work. *Sci Rep.* 2017;7(1):16310.
18. Kervezee L, Cuesta M, Cermakian N, Boivin DB. Simulated night shift work induces circadian misalignment of the human peripheral blood mononuclear cell transcriptome. *Proc Natl Acad Sci U S A.* 2018;115(21):5540-5545.
19. Damiola F, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F, Schibler U. Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev.* 2000;14(23):2950-2961.
20. Hara R, Wan K, Wakamatsu H, et al. Restricted feeding entrains liver clock without participation of the suprachiasmatic nucleus. *Genes Cells.* 2001;6(3):269-278.
21. Le Minh N, Damiola F, Tronche F, Schütz G, Schibler U. Glucocorticoid hormones inhibit food-induced phase-shifting of peripheral circadian oscillators. *Embo j.* 2001;20(24):7128-7136.
22. Thaiss CA, Zeevi D, Levy M, et al. Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell.*159(3):514-529.

23. Zarrinpar A, Chaix A, Yooseph S, Panda S. Diet and feeding pattern affect the diurnal dynamics of the gut microbiome. *Cell Metab.*20(6):1006-1017.
24. Liang X, Bushman FD, FitzGerald GA. Rhythmicity of the intestinal microbiota is regulated by gender and the host circadian clock. *Proc Natl Acad Sci U S A.*112(33):10479-10484.
25. Leone V, Gibbons SM, Martinez K, et al. Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. *Cell Host Microbe.* 2015;17(5):681-689.
26. Segers A, Desmet L, Sun S, Verbeke K, Tack J, Depoortere I. Night-time feeding of Bmal1-/- mice restores SCFA rhythms and their effect on ghrelin. *J Endocrinol.* 2020;245(1):155-164.
27. Tahara Y, Yamazaki M, Sukigara H, et al. Gut Microbiota-Derived Short Chain Fatty Acids Induce Circadian Clock Entrainment in Mouse Peripheral Tissue. *Sci Rep.* 2018;8(1):1395.
28. LeSauter J, Hoque N, Weintraub M, Pfaff DW, Silver R. Stomach ghrelin-secreting cells as food-entrainable circadian clocks. *Proc Natl Acad Sci U S A.* 2009;106(32):13582-13587.
29. Laermans J, Vancleef L, Tack J, Depoortere I. Role of the clock gene Bmal1 and the gastric ghrelin-secreting cell in the circadian regulation of the ghrelin-GOAT system. *Sci Rep.* 2015;5:16748.
30. Yildiz BO, Suchard MA, Wong ML, McCann SM, Licinio J. Alterations in the dynamics of circulating ghrelin, adiponectin, and leptin in human obesity. *Proc Natl Acad Sci U S A.* 2004;101(28):10434-10439.
31. Shiiya T, Nakazato M, Mizuta M, et al. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J Clin Endocrinol Metab.* 2002;87(1):240-244.

32. Qian J, Morris CJ, Caputo R, Garaulet M, Scheer FAJL. Ghrelin is impacted by the endogenous circadian system and by circadian misalignment in humans. *International journal of obesity (2005).* 2019;43(8):1644-1649.

33. Gil-Lozano M, Mingomataj EL, Wu WK, Ridout SA, Brubaker PL. Circadian secretion of the intestinal hormone GLP-1 by the rodent L cell. *Diabetes.* 2014;63(11):3674-3685.

34. Gil-Lozano M, Hunter PM, Behan LA, Gladanac B, Casper RF, Brubaker PL. Short-term sleep deprivation with nocturnal light exposure alters time-dependent glucagon-like peptide-1 and insulin secretion in male volunteers. *Am J Physiol Endocrinol Metab.* 2016;310(1):E41-50.

35. Elliott RM, Morgan LM, Tredger JA, Deacon S, Wright J, Marks V. Glucagon-like peptide-1 (7-36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. *J Endocrinol.* 1993;138(1):159-166.

36. Biancolin AD, Martchenko A, Mitova E, et al. The core clock gene, Bmal1, and its downstream target, the SNARE regulatory protein secretagogin, are necessary for circadian secretion of glucagon-like peptide-1. *Molecular metabolism.* 2020;31:124-137.

37. Yu X, Rollins D, Ruhn KA, et al. TH17 cell differentiation is regulated by the circadian clock. *Science.* 2013;342(6159):727-730.

38. Gibbs JE, Blaikley J, Beesley S, et al. The nuclear receptor REV-ERBalpha mediates circadian regulation of innate immunity through selective regulation of inflammatory cytokines. *Proc Natl Acad Sci U S A.* 2012;109(2):582-587.

39. Keller M, Mazuch J, Abraham U, et al. A circadian clock in macrophages controls inflammatory immune responses. *Proc Natl Acad Sci U S A.* 2009;106(50):21407-21412.

40. Scheiermann C, Kunisaki Y, Lucas D, et al. Adrenergic nerves govern circadian leukocyte recruitment to tissues. *Immunity.* 2012;37(2):290-301.

41. He W, Holtkamp S, Hergenhan SM, et al. Circadian Expression of Migratory Factors Establishes Lineage-Specific Signatures that Guide the Homing of Leukocyte Subsets to Tissues. *Immunity.* 2018;49(6):1175-1190.e1177.
42. Sletvold O. Circadian rhythms of peripheral blood leukocytes in aging mice. *Mech Ageing Dev.* 1987;39(3):251-261.
43. Summa KC, Voigt RM, Forsyth CB, et al. Disruption of the Circadian Clock in Mice Increases Intestinal Permeability and Promotes Alcohol-Induced Hepatic Pathology and Inflammation. *PloS one.* 2013;8(6):e67102-e67102.
44. Gutierrez JA, Solenberg PJ, Perkins DR, et al. Ghrelin octanoylation mediated by an orphan lipid transferase. *Proc Natl Acad Sci U S A.* 2008;105(17):6320-6325.
45. Yang J, Brown MS, Liang G, Grishin NV, Goldstein JL. Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell.* 2008;132(3):387-396.
46. Fonken LK, Aubrecht TG, Meléndez-Fernández OH, Weil ZM, Nelson RJ. Dim light at night disrupts molecular circadian rhythms and increases body weight. *Journal of biological rhythms.* 2013;28(4):262-271.
47. Kohsaka A, Laposky AD, Ramsey KM, et al. High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metab.* 2007;6(5):414-421.
48. Branecky KL, Niswender KD, Pendergast JS. Disruption of Daily Rhythms by High-Fat Diet Is Reversible. *PLoS One.* 2015;10(9):e0137970.
49. Pendergast JS, Branecky KL, Yang W, Ellacott KL, Niswender KD, Yamazaki S. High-fat diet acutely affects circadian organisation and eating behavior. *Eur J Neurosci.* 2013;37(8):1350-1356.
50. Yi CX, van der Vliet J, Dai J, Yin G, Ru L, Buijs RM. Ventromedial arcuate nucleus communicates peripheral metabolic information to the suprachiasmatic nucleus. *Endocrinology.* 2006;147(1):283-294.

51. Krashes MJ, Shah BP, Koda S, Lowell BB. Rapid versus delayed stimulation of feeding by the endogenously released AgRP neuron mediators GABA, NPY, and AgRP. *Cell Metab.* 2013;18(4):588-595.

52. Cedernaes J, Huang W, Ramsey KM, et al. Transcriptional Basis for Rhythmic Control of Hunger and Metabolism within the AgRP Neuron. *Cell Metab.* 2019;29(5):1078-1091.e1075.

53. Tan K, Knight ZA, Friedman JM. Ablation of AgRP neurons impairs adaption to restricted feeding. *Molecular metabolism.* 2014;3(7):694-704.

54. Riediger T, Traebert M, Schmid HA, Scheel C, Lutz TA, Scharrer E. Site-specific effects of ghrelin on the neuronal activity in the hypothalamic arcuate nucleus. *Neurosci Lett.* 2003;341(2):151-155.

55. Abizaid A, Horvath TL. Ghrelin and the central regulation of feeding and energy balance. *Indian journal of endocrinology and metabolism.* 2012;16(Suppl 3):S617-S626.

56. NamKoong C, Kim MS, Jang BT, Lee YH, Cho YM, Choi HJ. Central administration of GLP-1 and GIP decreases feeding in mice. *Biochem Biophys Res Commun.* 2017;490(2):247-252.

57. Riediger T, Eisele N, Scheel C, Lutz TA. Effects of glucagon-like peptide 1 and oxyntomodulin on neuronal activity of ghrelin-sensitive neurons in the hypothalamic arcuate nucleus. *Am J Physiol Regul Integr Comp Physiol.* 2010;298(4):R1061-1067.

58. Segers A, Desmet L, Thijs T, Verbeke K, Tack J, Depoortere I. The circadian clock regulates the diurnal levels of microbial short-chain fatty acids and their rhythmic effects on colon contractility in mice. *Acta Physiol (Oxf).* 2019;225(3):e13193.

59. Govindarajan K, MacSharry J, Casey PG, Shanahan F, Joyce SA, Gahan CG. Unconjugated Bile Acids Influence Expression of Circadian Genes: A Potential Mechanism for Microbe-Host Crosstalk. *PLoS One.* 2016;11(12):e0167319.

60. Ferrell JM, Chiang JYL. Short-term circadian disruption impairs bile acid and lipid homeostasis in mice. *Cellular and molecular gastroenterology and hepatology.* 2015;1(6):664-677.

61. Zhang YK, Guo GL, Klaassen CD. Diurnal variations of mouse plasma and hepatic bile acid concentrations as well as expression of biosynthetic enzymes and transporters. *PLoS One.* 2011;6(2):e16683.

62. Ma K, Xiao R, Tseng HT, Shan L, Fu L, Moore DD. Circadian dysregulation disrupts bile acid homeostasis. *PLoS One.* 2009;4(8):e6843.

63. Balsalobre A, Brown SA, Marcacci L, et al. Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science.* 2000;289(5488):2344-2347.

64. Fu L, Patel MS, Bradley A, Wagner EF, Karsenty G. The molecular clock mediates leptin-regulated bone formation. *Cell.* 2005;122(5):803-815.

65. Sun X, Dang F, Zhang D, et al. Glucagon-CREB/CRTC2 signaling cascade regulates hepatic BMAL1 protein. *J Biol Chem.* 2015;290(4):2189-2197.

66. Wang Q, Yin Y, Zhang W. Ghrelin Restores the Disruption of the Circadian Clock in Steatotic Liver. *Int J Mol Sci.* 2018;19(10).

67. Martchenko A, Oh RH, Wheeler SE, Gurges P, Chalmers JA, Brubaker PL. Suppression of circadian secretion of glucagon-like peptide-1 by the saturated fatty acid, palmitate. *Acta Physiol (Oxf).* 2018;222(4):e13007.

68. Anini Y, Brubaker PL. Role of leptin in the regulation of glucagon-like peptide-1 secretion. *Diabetes.* 2003;52(2):252-259.

69. Williams DL, Baskin DG, Schwartz MW. Leptin regulation of the anorexic response to glucagon-like peptide-1 receptor stimulation. *Diabetes.* 2006;55(12):3387-3393.

70. Scheer FA, Hilton MF, Mantzoros CS, Shea SA. Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc Natl Acad Sci U S A.* 2009;106(11):4453-4458.

71. Crispim CA, Waterhouse J, Damaso AR, et al. Hormonal appetite control is altered by shift work: a preliminary study. *Metabolism.* 2011;60(12):1726-1735.

72. Petrenko V, Philippe J, Dibner C. Time zones of pancreatic islet metabolism. *Diabetes Obes Metab.* 2018;20 Suppl 2:116-126.

73. Martchenko SE, Martchenko A, Cox BJ, et al. Circadian GLP-1 Secretion in Mice Is Dependent on the Intestinal Microbiome for Maintenance of Diurnal Metabolic Homeostasis. *Diabetes.* 2020;69(12):2589-2602.

74. Howick K, Griffin BT, Cryan JF, Schellekens H. From Belly to Brain: Targeting the Ghrelin Receptor in Appetite and Food Intake Regulation. *International journal of molecular sciences.* 2017;18(2):273.

75. Lin L, Nuotio-Antar AM, Ma X, Liu F, Fiorotto ML, Sun Y. Ghrelin receptor regulates appetite and satiety during aging in mice by regulating meal frequency and portion size but not total food intake. *The Journal of nutrition.* 2014;144(9):1349-1355.

76. Nakazato M, Murakami N, Date Y, et al. A role for ghrelin in the central regulation of feeding. *Nature.* 2001;409(6817):194-198.

77. Gonnissen HK, Rutters F, Mazuy C, Martens EA, Adam TC, Westerterp-Plantenga MS. Effect of a phase advance and phase delay of the 24-h cycle on energy metabolism, appetite, and related hormones. *Am J Clin Nutr.* 2012;96(4):689-697.

78. Stokes K, Cooke A, Chang H, Weaver DR, Breault DT, Karpowicz P. The Circadian Clock Gene BMAL1 Coordinates Intestinal Regeneration. *Cellular and molecular gastroenterology and hepatology.* 2017;4(1):95-114.

79. Laermans J, Broers C, Beckers K, et al. Shifting the circadian rhythm of feeding in mice induces gastrointestinal, metabolic and immune alterations which are influenced by ghrelin and the core clock gene Bmal1. *PLoS One.* 2014;9(10):e110176.

80. Deaver JA, Eum SY, Toborek M. Circadian Disruption Changes Gut Microbiome Taxa and Functional Gene Composition. *Frontiers in microbiology.* 2018;9:737-737.

81. Amara J, Saliba Y, Hajal J, et al. Circadian Rhythm Disruption Aggravates DSS-Induced Colitis in Mice with Fecal Calprotectin as a Marker of Colitis Severity. *Dig Dis Sci.* 2019;64(11):3122-3133.

82. Hemmeryckx B, Himmelreich U, Hoylaerts MF, Lijnen HR. Impact of clock gene Bmal1 deficiency on nutritionally induced obesity in mice. *Obesity (Silver Spring).* 2011;19(3):659-661.

83. Vandesompele J, De Preter K, Pattyn F, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol.* 2002;3(7):RESEARCH0034.

84. Janssen S, Laermans J, Verhulst PJ, Thijs T, Tack J, Depoortere I. Bitter taste receptors and alpha-gustducin regulate the secretion of ghrelin with functional effects on food intake and gastric emptying. *Proc Natl Acad Sci U S A.* 2011;108(5):2094-2099.

85. Nelson W, Tong YL, Lee JK, Halberg F. Methods for cosinor-rhythmometry. *Chronobiologia.* 1979;6(4):305-323.

TABLES

Table 1. Cosinor values of all measured parameters.

			Condition	P-value	Acrophase (h)	Acrophase	P-value Acrophase
Faecal SCFA	Acetate		Control	ns	NA	NA	NA
			Jetlag	ns	NA		
	Propionate		Control	<0.05	4:47 (±1:21)	5:19	<0.01
			Jetlag	<0.05	10:05 (±1:26)		
	Butyrate		Control	<0.01	3:04 (±0:58)	2:43	<0.05
			Jetlag	<0.01	5:47 (±1:01)		
Distal Colonic mRNA Expression	Circadian Clock	*Arntl*	Control	<0.001	2:19 (±0:10)	4:20	<0.001
			Jetlag	<0.001	6:39 (±0:19)		
		Clock	Control	<0.001	2:43 (±0:37)	2:10	<0.05
			Jetlag	<0.001	4:52 (±0:37)		
		Rev-erbα	Control	<0.001	10:37 (±0:12)	4:53	<0.01
			Jetlag	<0.001	15:31 (±0:26)		
		Per2	Control	<0.001	17:43 (±0:20)	4:49	<0.001
			Jetlag	<0.001	22:32 (±0:32)		
	GLP-1	*Proglucagon*	Control	<0.001	5:07 (±0:49)	NA	NA
			Jetlag	ns	NA		
	cytokines	*Tnfα*	Control	<0.01	8:31 (±1:11)	NA	NA
			Jetlag	ns	NA		
	Receptors	*Ffar2*	Control	<0.05	4:06 (±1:34)	NA	NA
			Jetlag	ns	NA		
	tight junction markers	*Occludin*	Control	<0.01	3:36 (±:h04)	NA	NA
			Jetlag	<0.05	3:29 (±1:19)		
		Claudin-1	Control	ns	NA	NA	NA
			Jetlag	ns	NA		
		Tjp1	Control	ns	NA	NA	NA
			Jetlag	ns	NA		
Stomach Mucosa mRNA Expression	Circadian Clock	*Arntl*	Control	<0.001	0:37 (±0:15)	4:44	<0.001
			Jetlag	<0.001	5:20 (±0:28)		
		Clock	Control	<0.001	22:22 (±0:40)	NA	NA
			Jetlag	ns	NA		
		Rev-erbα	Control	<0.001	9:37 (±0:10)	4:34	<0.001
			Jetlag	<0.001	14:11 (±0:22)		
	Ghrelin	*Ghrelin*	Control	<0.01	5:07 (±1:00)	NA	NA
			Jetlag	ns	NA		
		Goat	Control	<0.05	9:29 (±1:32)	NA	NA
			Jetlag	<0.05	10:33 (±1:20)		
Plasma	Ghrelin		Control	<0.05	14:33 (±1:17)	NA	NA
			Jetlag	<0.05	14:44 (±1:20)		
	GLP-1		Control	<0.01	16:41 (±0:59)	NA	NA
			Jetlag	<0.05	13:30 (±1:18)		

Table 2. Primers used in qRT-PCR

Gene	Forward primer	Reverse primer
TBP	5’ AGGATGCTCTAGGGAAGAT 3’	5’ TGAATAGGCTGTGGAGTAAGT 3’
Hmbs	5’ CTGAAGGATGTGCCTACCATAC 3’	5’ AAGGTTTCCAGGGTCTTTCC 3’
Cycloph	5’ GGAGATGGCACAGGAGGAAA 3’	5’ CCCGTAGTGCTTCAGCTTGAA 3’
GAPDH	5’ GTGTCCGTCGTGGATCTGA 3’	5’ CCTGCTTCACCACCTTCTTG 3’
β-Actin	5’ GATCTGGCACCACACCTTCTAC 3’	5’ TGGATGGCTACGTACATGGCTG 3’
Arntl	5’ CGTTTCTCGACACGCAATAGAT 3’	5’ TCCTGTGGTAGATACGCCAAAA 3’
Reverbα	5’ CCCTGGACTCCAATAACAACACA 3’	5’ GCCATTGGAGCTGTCACTGTAG 3’
Clock	5’ TCTACAGAAGAGCATTGATTTTTTGC 3’	5’ TCATTACTAAGGAATGTGGGTTTCC 3’
Per2	5’ GATGACAGAGGCAGAGCACAAC 3’	5’ TTTGTGTGCGTCAGCTTTGG 3’
Proglucagon	5’ GAGGAGAACCCCAGATCATTCC 3’	5’ GTGGCGTTTGTCTTCATTCATC 3’
Ffar2	5’ CCCTGTGCACATCCTCCTGC 3’	5’ GCGTTCCATGCTGATGCCCG 3’
Ghrelin	5’ CCAGAGGACAGAGGACAAGC 3’	5’ ACATCGAAGGGAGCATTGAA 3’
Goat	5’ ATTTGTGAAGGGAAGGTGGAG 3’	5’ CAGGAGAGCAGGGAAAAAGAG 3’
Tnfα	5’ TCTTCTCATTCCTGCTTGTGG 3’	5’ CACTTGGTGGTTTGCTACGA 3’
Ocln	5’ GACTGGGTCAGGGAATATCCACC 3’	5’ AGCAGCAGCCATGTACTCTTCAC 3’
Cldn1	5’ AGACCTGGATTTGCATCTTGGTG 3’	5’ TGCAACATAGGCAGGACAAGAGTTA 3’
Tjp1	5’ TCACGATCTCCTGACCAACG 3’	5’ GGCTGACGGGTAAATCCACA 3’

FIGURE LEGENDS

Figure 1. **Jetlag promotes body weight gain and shifts the day/night food intake pattern without affecting total daily caloric intake.** (a) Percentage body weight gain, (b) total 24h caloric intake and (c) day/night food intake of control (n = 48) and jetlagged (n = 48) mice. Data are presented as mean ± SEM. †: P < 0.01; ‡: P < 0.001.

Figure 2. **Jetlag shifts faecal propionate and butyrate concentrations.** (a, b, c) Faecal acetate, propionate and butyrate concentrations in the distal colon of control and jetlagged mice (n = 8 mice per condition and time point). The fitted cosine curve determined by cosinor analysis (period = 24 hours) is shown for propionate and butyrate in both control (grey line) and jetlagged (dashed black line) mice. The dark phase is shaded grey. The direction of the shift due to the jetlag is indicated by a black arrow. ns: not significant.

Figure 3. **The shift in circadian clock gene expression in the mucosa of the colon and stomach parallels the shift in faecal SCFAs.** (a, b, c) *Arntl*, *Clock* and *Reverbα* mRNA expression in the distal colonic mucosa of control and jetlagged mice (n = 8 mice per condition and time point). (d) Acrophase plot of faecal SCFA concentrations and circadian clock gene expression in the distal colonic mucosa in control and jetlagged mice. (e) Acrophase plot of circadian clock gene expression in the mucosa of the stomach and the colon in control and jetlagged mice. The dark phase is shaded grey. The direction of the shift of the fitted cosine curve due to jetlag is indicated by a black arrow.

Figure 4. **Graphical representation of the acrophases of all measured parameters in both control and jetlagged mice.** Upper figure: Control mice; Lower figure: Jetlagged mice. The dark phase (=active phase) is shaded blue. * in the lower figure indicates a significant shift/loss in acrophase between control and jetlagged mice.

Figure 5. **Faecal concentrations of SCFAs shift the circadian clock mRNA expression in primary colonic crypts.** (a) *Arntl*, (b) *Reverbα* and (c) *Per2* mRNA expression in synchronized (200 nM dexamethasone, 2h) primary colonic crypts (n = 4 mice per time point) stimulated with DMEM (grey line) or a mixture of SCFAs (24 mM) (dashed black line). The direction of the shift of the fitted cosine curve due to SCFA treatment is indicated by a black arrow.

Figure 6. **Jetlag abolishes the rhythmicity in *Proglucagon* and *Ffar2* mRNA expression in the distal colonic mucosa and dampens plasma GLP-1 concentrations.** (a, b) *Proglucagon* and *Ffar2* mRNA expression in the distal colonic mucosa and (c) plasma GLP-1 levels of control and jetlagged mice (n = 8 mice per condition and time point). (d) Blood glucose levels in control and jetlagged mice (n = 3-8 per condition and time point). The fitted cosine curve determined by cosinor analysis (period = 24 hours) is shown in both control (grey line) and jetlagged (dashed black line) mice. The dark phase is shaded grey.

Figure 7. **Jetlag abrogates *Ghrelin* mRNA expression, but not *Goat* mRNA expression and plasma ghrelin levels.** (a, b) *Ghrelin* and *Goat* mRNA expression in the gastric mucosa and (c) Plasma ghrelin concentrations in control and jetlagged mice (n = 8 mice per condition and time point). The fitted cosine curve determined by cosinor analysis (period = 24 hours) is shown in both control (grey line) and jetlagged (dashed black line) mice. The dark phase is shaded grey. ns = not significant.

Figure 8. ***Ghrelin* and *Ffar2* mRNA expression, but not *Proglucagon* mRNA expression, in the distal colonic mucosa is regulated by the circadian clock.** (a, b, c) *Ghrelin*, *Ffar2* and *Proglucagon* mRNA expression in the distal colonic mucosa of WT and *Arntl*$^{-/-}$ mice at ZT4 and ZT16. n = 8 per genotype and time point *: $P < 0.05$ ZT4 vs ZT16; †: $P < 0.01$ Interaction effect (Genotype x ZT).

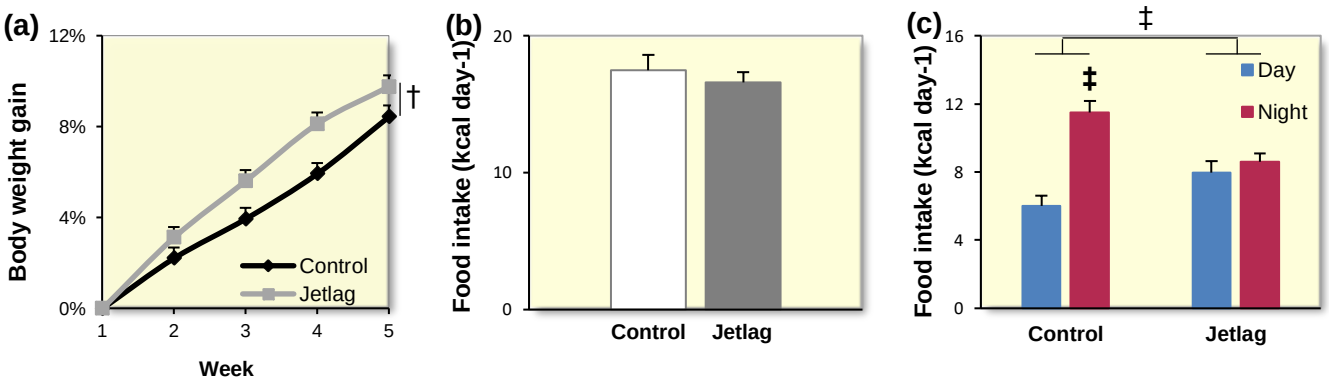
Figure 9. **Jetlag affects the rhythmicity of *Tnfα* expression but not of tight junction proteins.** (a, b, c) *Tnfα*, *Ocln* and *Cldn1* mRNA expression in the distal colonic mucosa of control and jetlagged mice (n = 8 mice per condition and time point). The fitted cosine curve

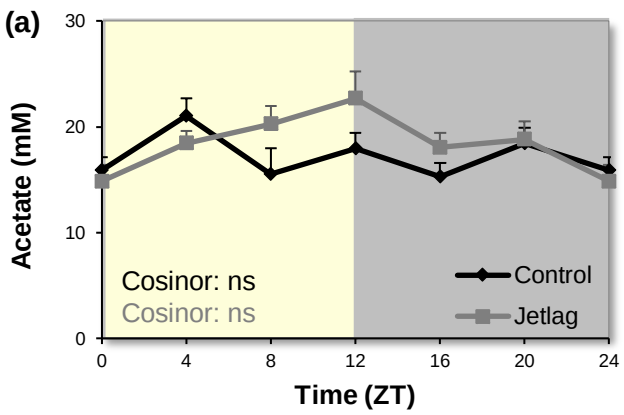
determined by cosinor analysis (period = 24 hours) is shown in both control (grey line) and jetlagged (dashed black line) mice. The dark phase is shaded grey. ns = not significant.

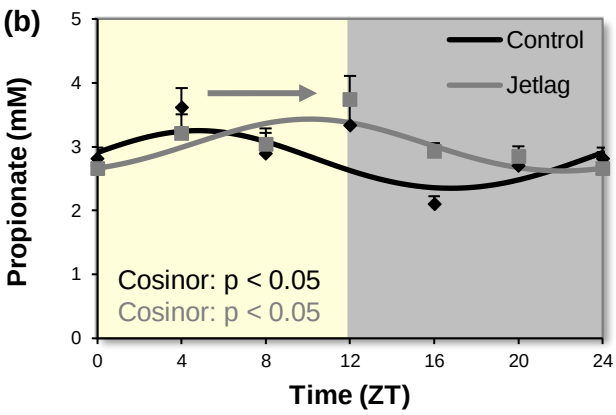
Figure S1. **Jetlag shifts *Per2* mRNA expression in distal colonic mucosa**. *Per2* mRNA expression in distal colonic mucosa of control and jetlagged mice (n = 8 mice per condition and time point). The dark phase is shaded grey. The direction of the shift of the fitted cosine curve due to the jetlag is indicated by a black arrow.

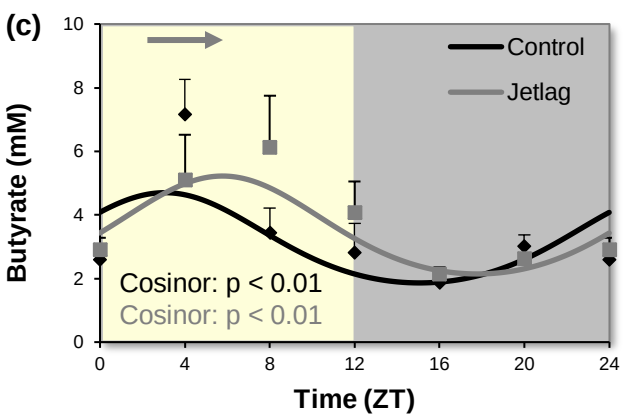
Figure S2. **Schematic representation of the jetlag model.** The light phase is represented in white, the dark phase is represented in black.

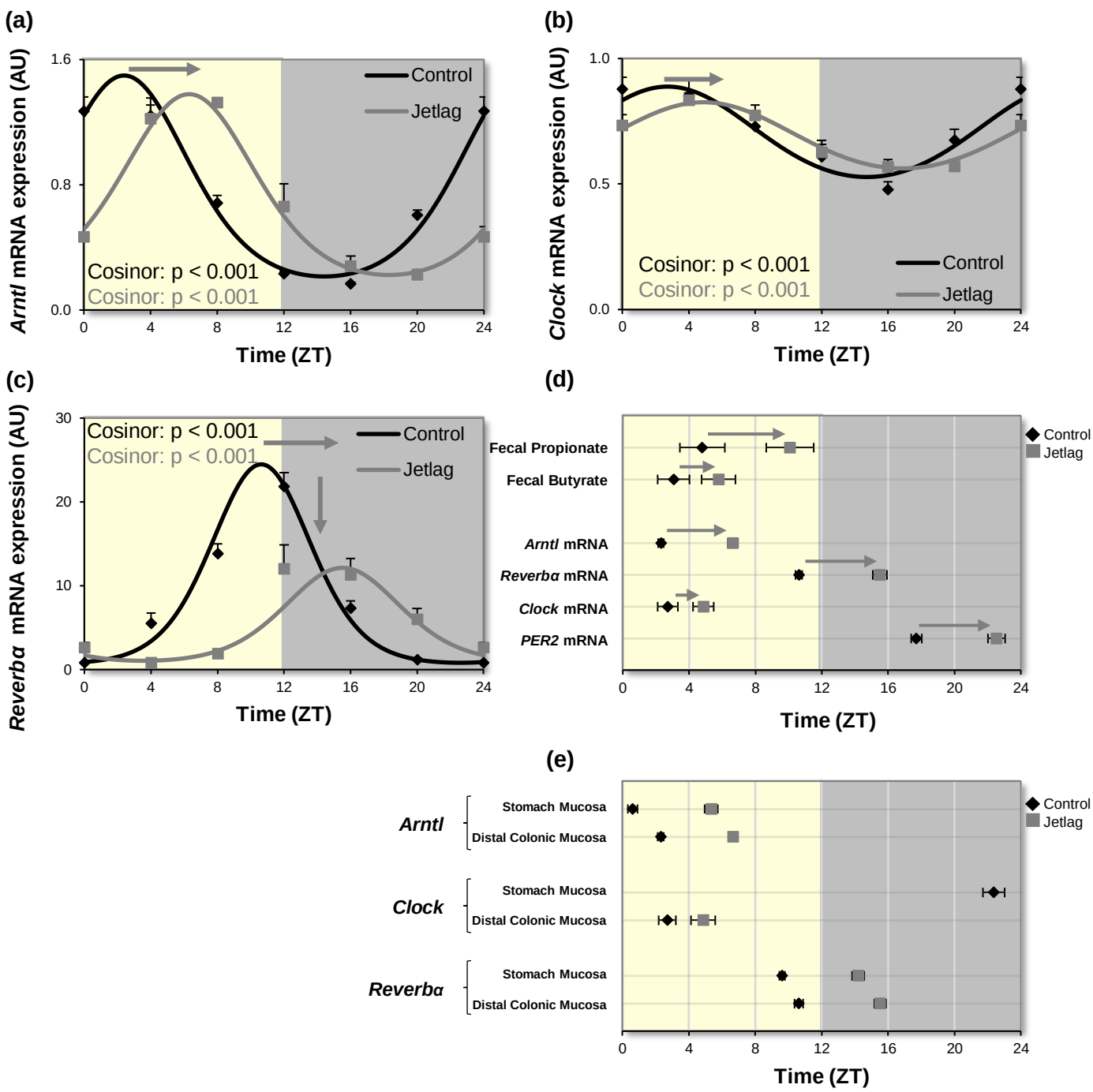
Figure S3. **Representative pictures of the primary colonic crypts.** (a) at the time of isolation and (b) after synchronization 20h after isolation.

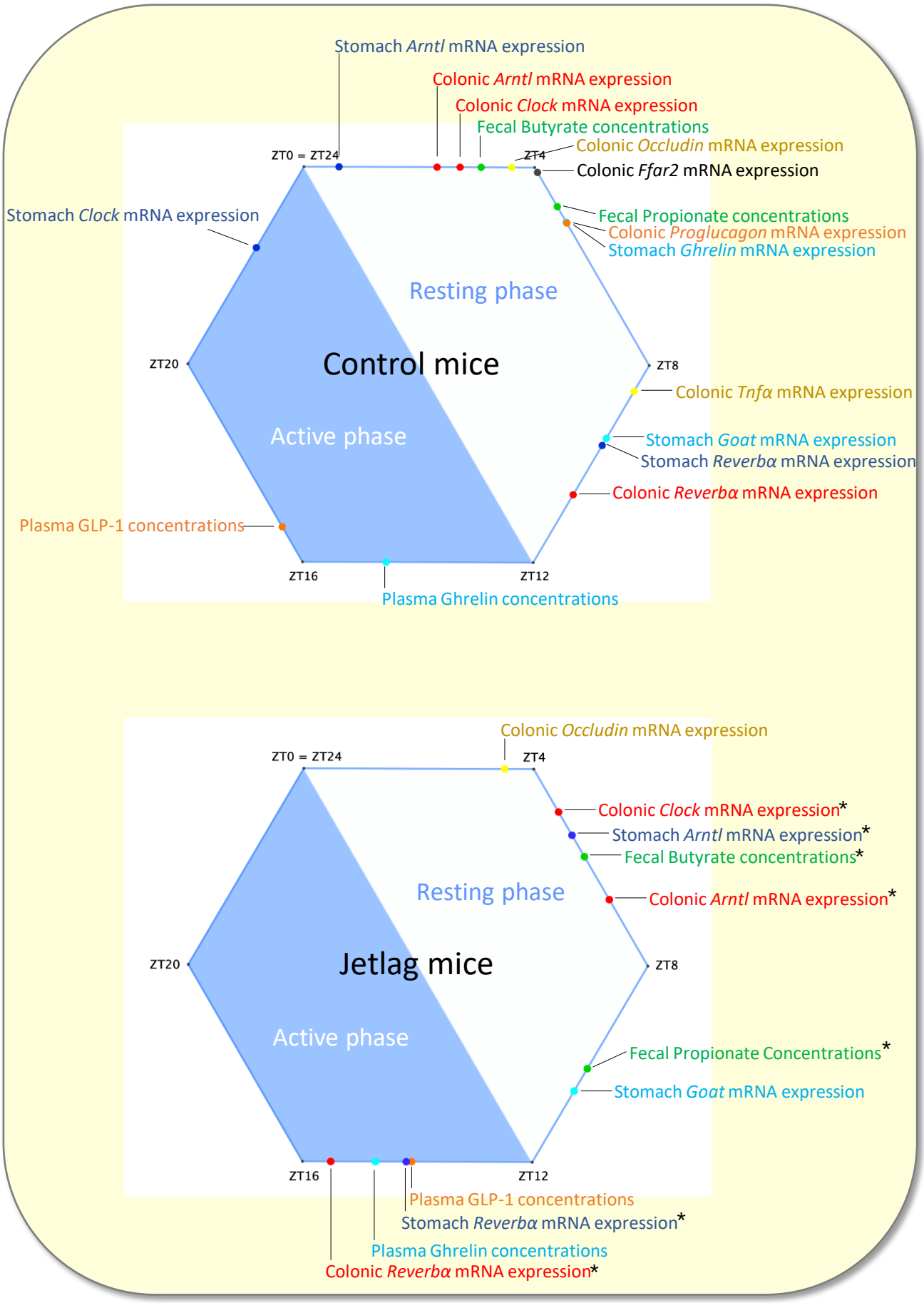

(a)
Body weight gain
12%
8%
4%
0%
1
2
3
4
5
Week
Control
Jetlag
†
(b)
Food intake (kcal day-1)
20
10
0
Control
Jetlag
(c)
Food intake (kcal day-1)
16
12
8
4
0
‡
‡
Day
Night
Control
Jetlag

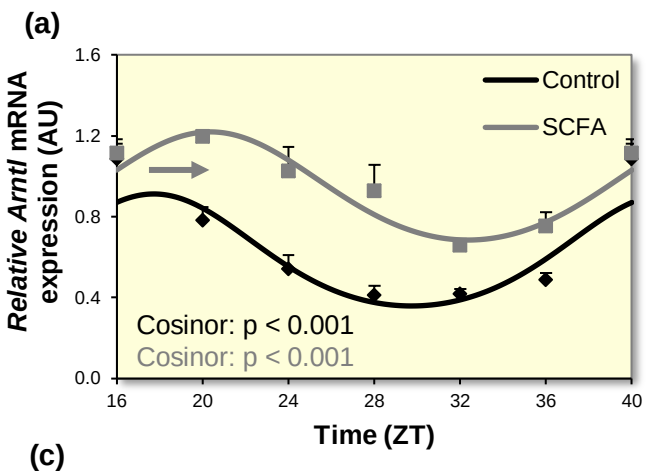

(a)
Acetate (mM)
30
20
10
0
0
4
8
12
16
20
24
Time (ZT)
Cosinor: ns
Cosinor: ns
Control
Jetlag

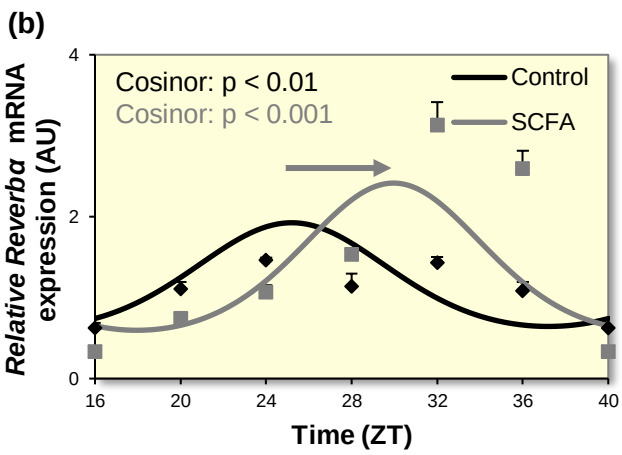

(b)
Propionate (mM)
5
4
3
2
1
0
0
4
8
12
16
20
24
Time (ZT)
Cosinor: p < 0.05
Cosinor: p < 0.05
Control
Jetlag

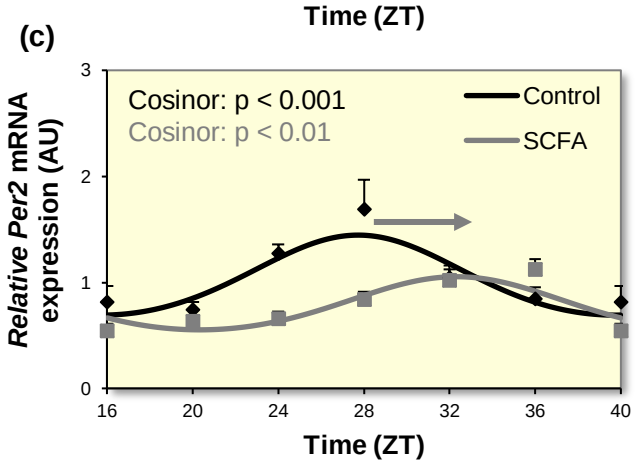

(c)
Butyrate (mM)
10
8
6
4
2
0
0
4
8
12
16
20
24
Time (ZT)
Cosinor: p < 0.01
Cosinor: p < 0.01
Control
Jetlag

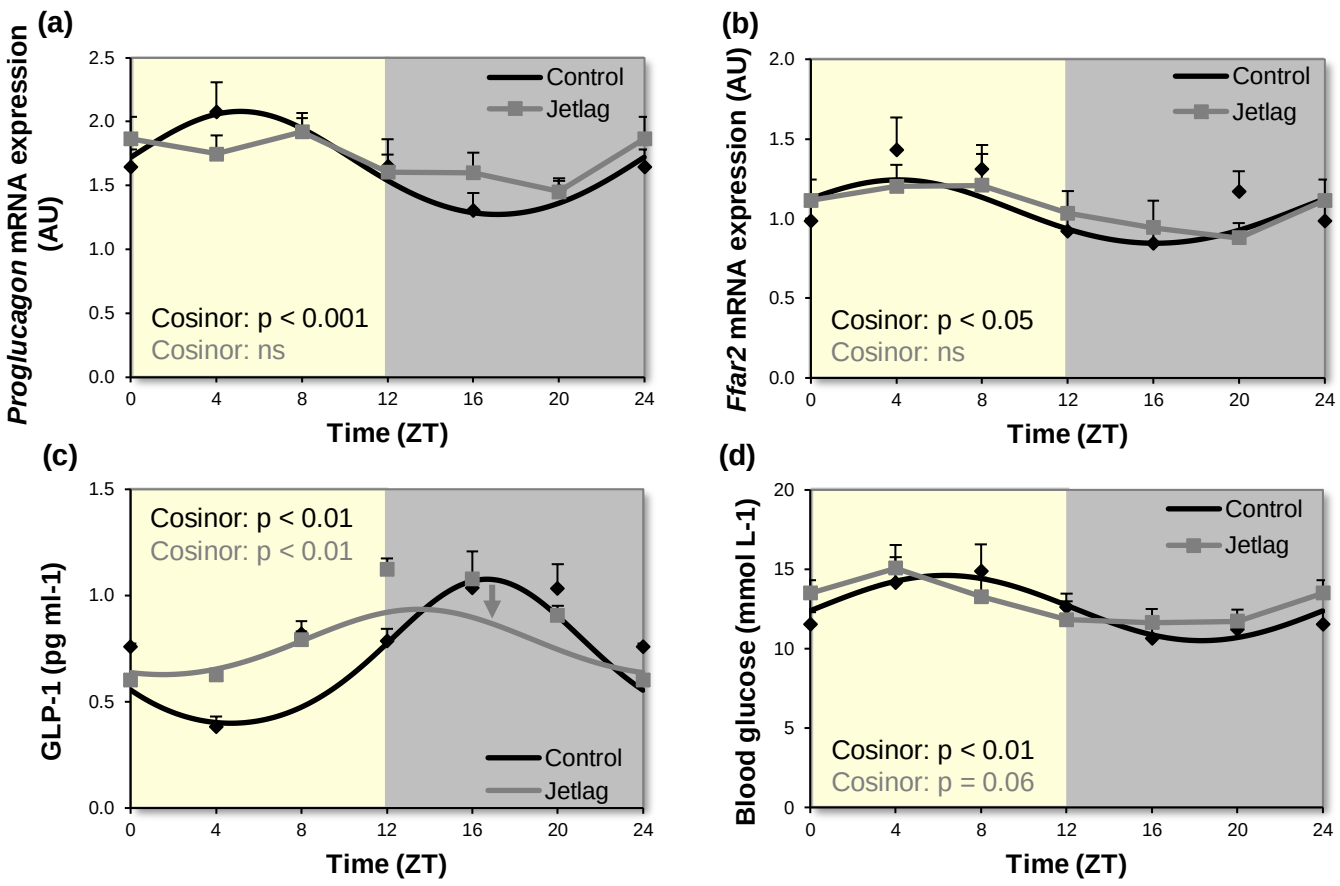

(a)
Arntl mRNA expression (AU)
1.6
0.8
0.0
Control
Jetlag
Cosinor: p < 0.001
Cosinor: p < 0.001
0
4
8
12
16
20
24
Time (ZT)
(b)
Clock mRNA expression (AU)
1.0
0.5
0.0
Cosinor: p < 0.001
Cosinor: p < 0.001
Control
Jetlag
Time (ZT)
(c)
Reverbα mRNA expression (AU)
30
20
10
0
Cosinor: p < 0.001
Cosinor: p < 0.001
Control
Jetlag
Time (ZT)
(d)
Fecal Propionate
Fecal Butyrate
Arntl mRNA
Reverbα mRNA
Clock mRNA
PER2 mRNA
Control
Jetlag
Time (ZT)
(e)
Arntl
Clock
Reverbα
Stomach Mucosa
Distal Colonic Mucosa
Control
Jetlag
Time (ZT)

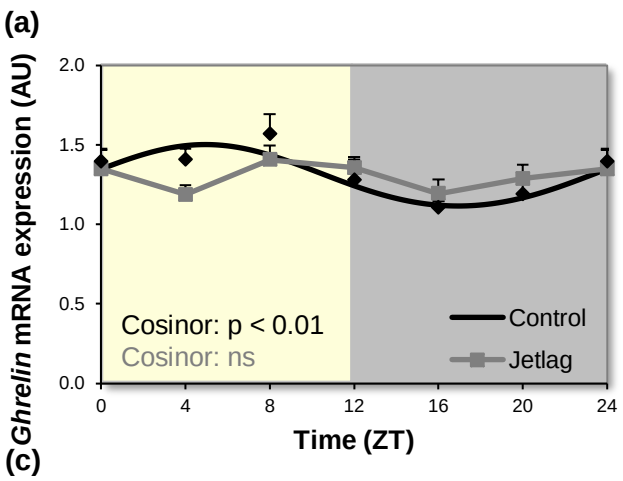

Stomach *Arntl* mRNA expression
Colonic *Arntl* mRNA expression
Colonic *Clock* mRNA expression
Fecal Butyrate concentrations
Colonic *Occludin* mRNA expression
Colonic *Ffar2* mRNA expression
Fecal Propionate concentrations
Colonic *Proglucagon* mRNA expression
Stomach *Ghrelin* mRNA expression
Stomach *Clock* mRNA expression
ZT0 = ZT24
ZT4
ZT8
ZT12
ZT16
ZT20
Resting phase
Control mice
Active phase
Colonic *Tnfα* mRNA expression
Stomach *Goat* mRNA expression
Stomach *Reverbα* mRNA expression
Colonic *Reverbα* mRNA expression
Plasma GLP-1 concentrations
Plasma Ghrelin concentrations
Colonic *Occludin* mRNA expression
ZT0 = ZT24
ZT4
Colonic *Clock* mRNA expression*
Stomach *Arntl* mRNA expression*
Fecal Butyrate concentrations*
Resting phase
Colonic *Arntl* mRNA expression*
ZT20
Jetlag mice
ZT8
Active phase
Fecal Propionate Concentrations*
Stomach *Goat* mRNA expression
ZT16
ZT12
Plasma GLP-1 concentrations
Stomach *Reverbα* mRNA expression*
Plasma Ghrelin concentrations
Colonic *Reverbα* mRNA expression*

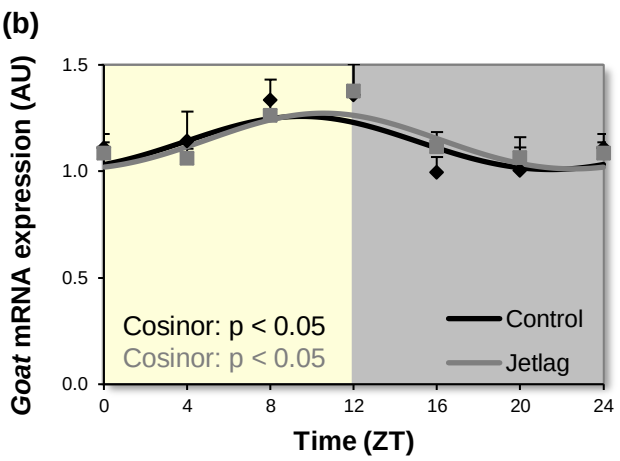

(a)
Relative Arntl mRNA expression (AU)
1.6
1.2
0.8
0.4
0.0
16
20
24
28
32
36
40
Time (ZT)
Control
SCFA
Cosinor: p < 0.001
Cosinor: p < 0.001

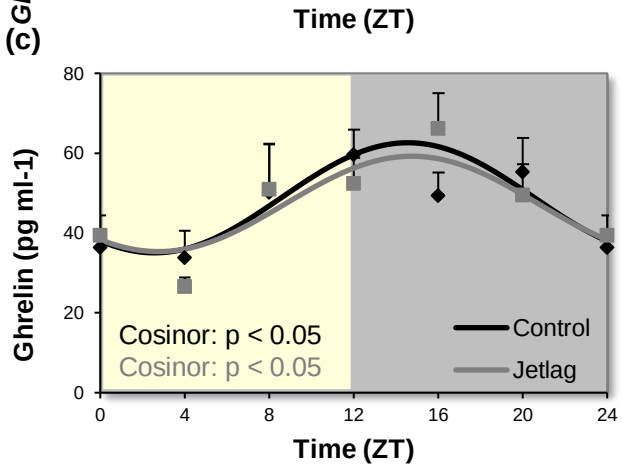

(b)
Relative Reverbα mRNA expression (AU)
4
2
0
16
20
24
28
32
36
40
Time (ZT)
Control
SCFA
Cosinor: p < 0.01
Cosinor: p < 0.001

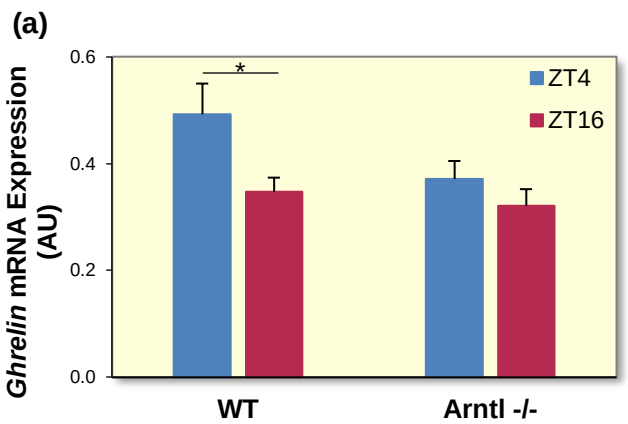

(c)
Relative Per2 mRNA expression (AU)
3
2
1
0
16
20
24
28
32
36
40
Time (ZT)
Control
SCFA
Cosinor: p < 0.001
Cosinor: p < 0.01

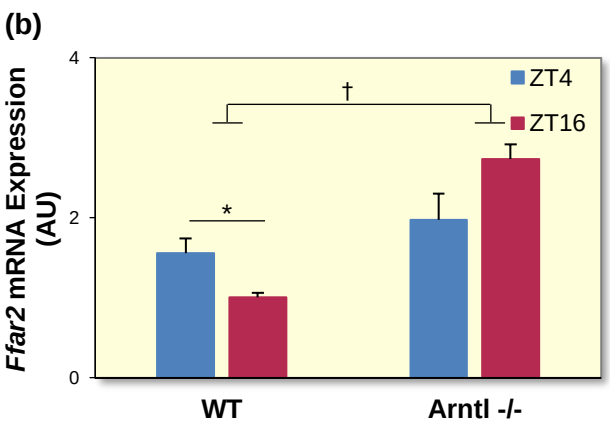

(a)
Proglucagon mRNA expression (AU)
Control
Jetlag
Cosinor: p < 0.001
Cosinor: ns
Time (ZT)
(b)
Ffar2 mRNA expression (AU)
Control
Jetlag
Cosinor: p < 0.05
Cosinor: ns
Time (ZT)
(c)
GLP-1 (pg ml-1)
Cosinor: p < 0.01
Cosinor: p < 0.01
Control
Jetlag
Time (ZT)
(d)
Blood glucose (mmol L-1)
Control
Jetlag
Cosinor: p < 0.01
Cosinor: p = 0.06
Time (ZT)

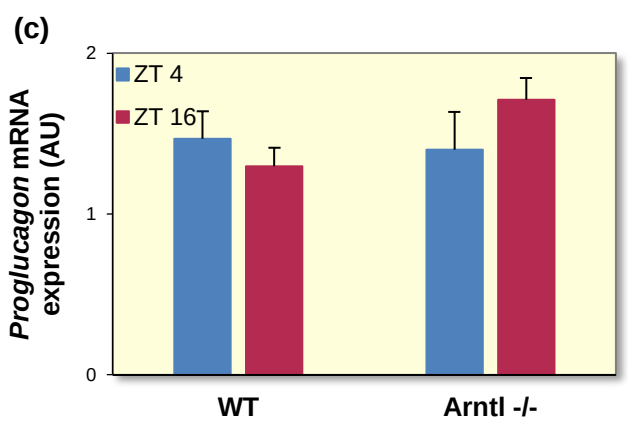

(a)
Ghrelin mRNA expression (AU)
2.0
1.5
1.0
0.5
0.0
0
4
8
12
16
20
24
Time (ZT)
Cosinor: p < 0.01
Cosinor: ns
Control
Jetlag

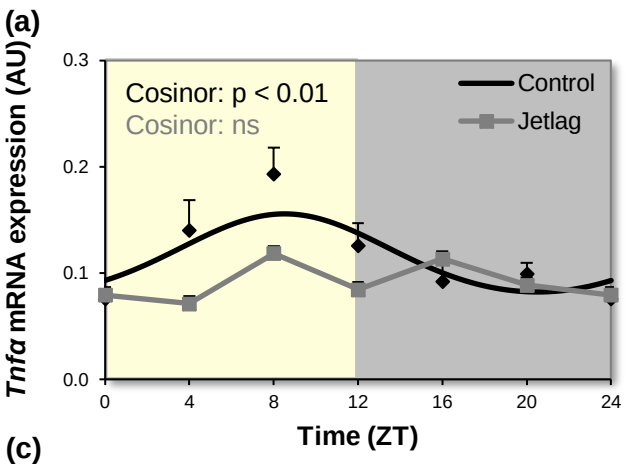

(b)
Goat mRNA expression (AU)
1.5
1.0
0.5
0.0
0
4
8
12
16
20
24
Time (ZT)
Cosinor: p < 0.05
Cosinor: p < 0.05
Control
Jetlag

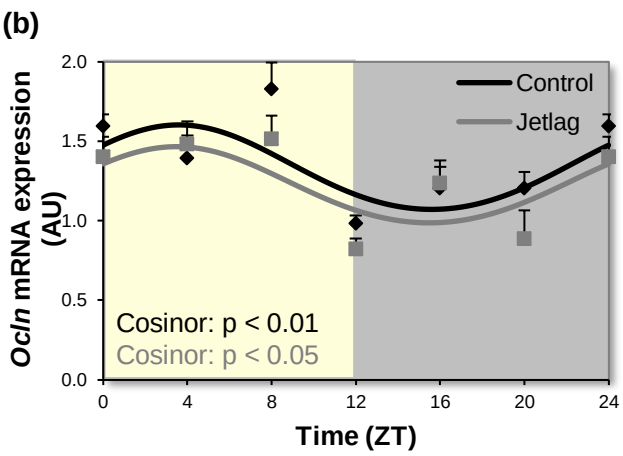

(c)
Ghrelin (pg ml-1)
80
60
40
20
0
0
4
8
12
16
20
24
Time (ZT)
Cosinor: p < 0.05
Cosinor: p < 0.05
Control
Jetlag

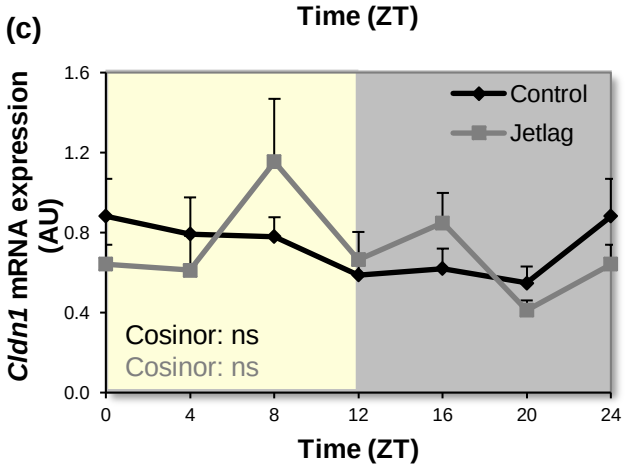

(a)
Ghrelin mRNA Expression (AU)
0.6
0.4
0.2
0.0
*
ZT4
ZT16
WT
Arntl -/-

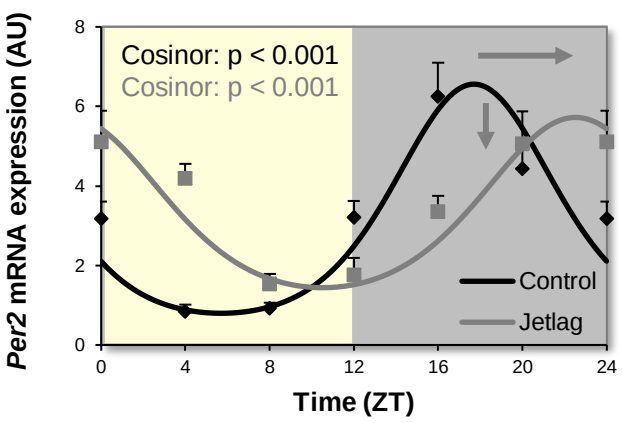

(b)
Ffar2 mRNA Expression (AU)
4
2
0
†
*
ZT4
ZT16
WT
Arntl -/-

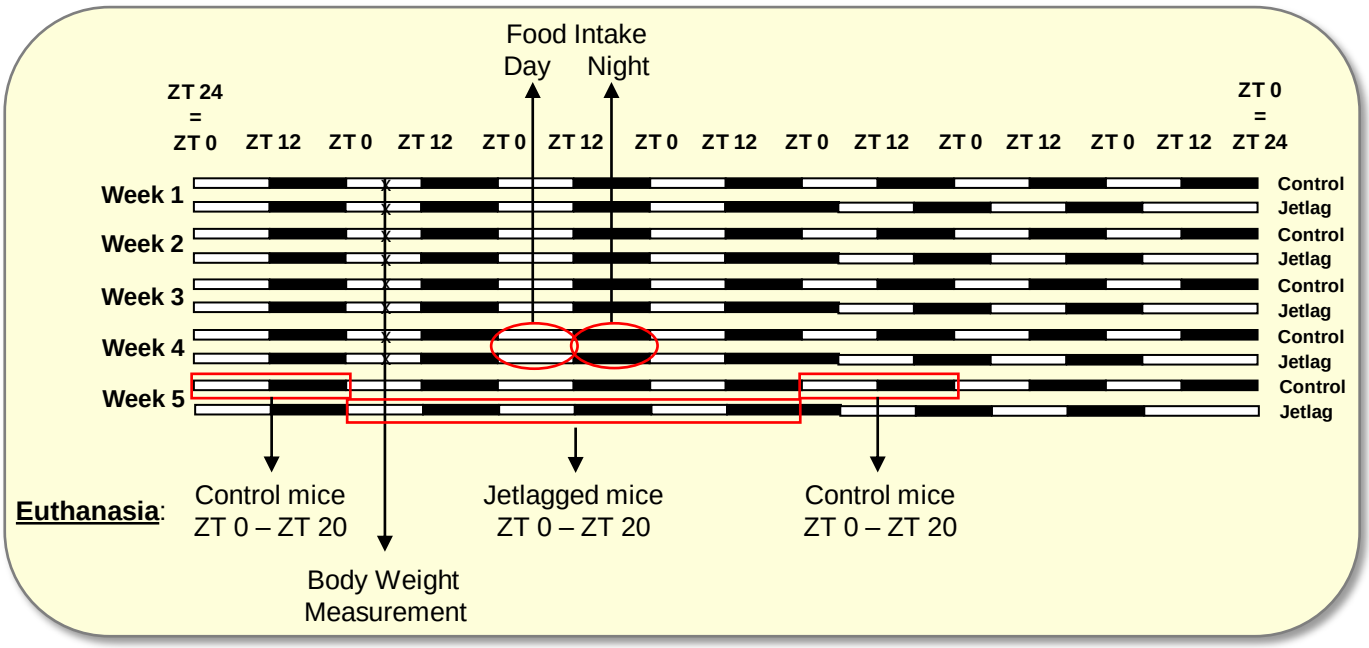

(c)
Proglucagon mRNA expression (AU)
2
1
0
ZT 4
ZT 16
WT
Arntl -/-


(a)
Tnfα mRNA expression (AU)
0.3
0.2
0.1
0.0
Cosinor: p < 0.01
Cosinor: ns
Control
Jetlag
0
4
8
12
16
20
24
Time (ZT)

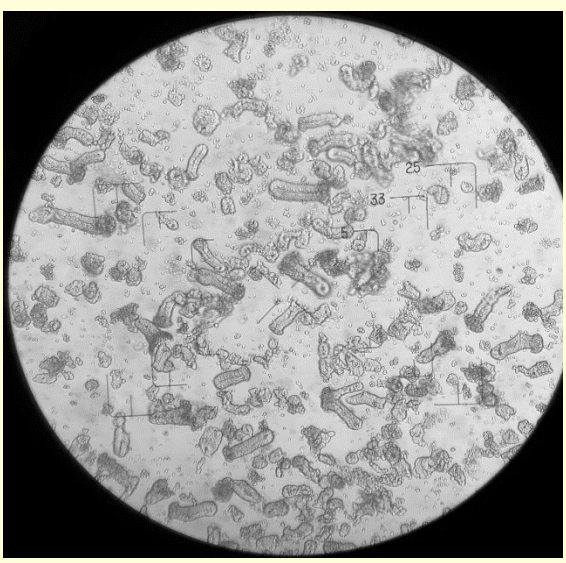

(b)
Ocln mRNA expression (AU)
2.0
1.5
1.0
0.5
0.0
Control
Jetlag
Cosinor: p < 0.01
Cosinor: p < 0.05
0
4
8
12
16
20
24
Time (ZT)


(c)
Cldn1 mRNA expression (AU)
1.6
1.2
0.8
0.4
0.0
Control
Jetlag
Cosinor: ns
Cosinor: ns
0
4
8
12
16
20
24
Time (ZT)


Cosinor: p < 0.001
Cosinor: p < 0.001
Control
Jetlag
Per2 mRNA expression (AU)
Time (ZT)
0
2
4
6
8
0
4
8
12
16
20
24


Food Intake
Day
Night
ZT 24 = ZT 0
ZT 12
ZT 0
ZT 12
ZT 0
ZT 12
ZT 0
ZT 12
ZT 0
ZT 12
ZT 0
ZT 12
ZT 0
ZT 12
ZT 0 = ZT 24
Week 1
Week 2
Week 3
Week 4
Week 5
Control
Jetlag
Euthanasia:
Control mice
ZT 0 – ZT 20
Jetlagged mice
ZT 0 – ZT 20
Control mice
ZT 0 – ZT 20
Body Weight
Measurement

(a)



(b)