Calorie restriction effects on circadian rhythms in gene expression are sex dependent

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The rhythms in the expression of circadian clock genes are affected by calorie restriction (CR), a dietary paradigm known to increase lifespan. Many physiological effects of CR differ between males and females; here we investigated if the sex of animals affects the CR induced changes in the circadian rhythms. The liver expression of some circadian clock genes such as Bmal1 and three Periods (Per1, Per2 and Per3) and the effect of CR on the expression of these genes were sex independent, while the expression of Rev-Erb alpha, Ror gamma and both Cryptochromes (Cry1 and Cry2) genes was different between males and females. The effect of CR on Rev-Erb alpha, Ror gamma and Cry1 gene expression was sex dependent. The expression and the effects of CR were sex specific for several genes previously reported to be regulated by CR: Fmo3, Mup4, Serpina12 and Cyp4a12, while the expression of Cyp4a14a was sex independent. IGF signaling plays an important role in aging and CR effects. IGF-1 expression is regulated by CR and by the circadian clock, we found that rhythms in IGF-1 expression have sexual dimorphism. Our data provide molecular evidence that the sex of animals is an important modulator of circadian rhythms in gene expression and their response to CR.
previously that 30% CR affects the amplitude and absolute level of expression for several circadian clock genes. The effect was observed on both mRNA and protein levels. Importantly, CR fails to provide all physiological benefits including the effect on longevity in mice deficient for the circadian transcriptional factor BMAL1. Similarly, CR does not have full effects on the increase in lifespan in Drosophila flies with genetic ablation of some clock genes. Thus, circadian clocks might represent a conserved physiological system essential for CR mechanisms, which warrants a further study on interaction between circadian clocks and CR.

In the previous studies the effect of CR on clock gene expression was investigated only in male mice. The majority of the studies on circadian clocks and rhythms either did not compare males to females or did not include females into the experimental setup at all - due to a general bias in biomedical research. Recent data suggest that many effects of CR are sex-dependent and some circadian rhythms in behavior and physiology are different in males and females. Therefore, in the present report we compared the effect of CR on circadian rhythms in gene expression between male and female mice.

Results

Effect of sex on the circadian rhythms in expression of clock genes. In the previous study we found that the daily rhythms in the expression of Bmal1, Per1, Per2, Per3, Cry2 and Ror in the liver of male mice were significantly affected by CR in the liver of male mice. Male and female mice of the same age (starting at 3 months) were subjected to 30% CR for two subsequent months. The expression of circadian clock genes was assayed in the liver of CR and control animals, which had ad libitum (AL) access to food throughout the day. We found that in the liver of AL mice the expression of Bmal1 and Per1, Per2 and Per3 genes was similar between males and females, and there were no significant differences in the phase of the expression (see Fig. 1). CR significantly induced the expression of these genes: at ZT22 and ZT2 for Bmal1; at ZT10 and ZT14 for Per1; at ZT18 for Per2 and ZT10 for Per3 in both males and females (Fig. 1). While at some time points (ZT2 for Bmal1, ZT10 for Per1 and Per3) we observed a statistically significant difference between the expressions in males and females, the changes in the expression were still observed for both males and females and these changes were towards the same direction. Therefore, we concluded that the sex of animals might have some effects on the magnitude of the response but the expression of Bmal1, Per1, Per2 and Per3 to CR was similar in both sexes. CR did not affect the phase of gene expression because the food for the CR group was provided at ZT14 (2 hours after the light was off), which is the physiological feeding time for nocturnal rodents.

Figure 1. Sex independent expression and response to CR for some circadian clock genes. The daily rhythms in expression of mRNA for Bmal1 (a), Per1 (b), Per2 (c) and Per3 (d) in the liver: blue diamonds and dashed lines – AL male mice; red squares and solid lines – CR male mice, green triangles and dashed lines – AL female mice; purple x and solid lines – CR female mice. For all panels graphs are double plotted. Light is on at ZT0 and off at ZT12. a, b, c, d – statistically significant difference (p < 0.05) between indicated groups at particular time point. Open bars represent light and black bars represent dark phase of the day. Food for CR group was provided at ZT14. At every time of the day 3 mice of each sex were used for each diet.
The expression of Cry1, Cry2 and Rev-Erb α and Ror γ was significantly affected by the sex of the mice. In the AL group the expression of all four genes was significantly higher in the liver of female mice: at ZT18, ZT22 and ZT2 for Cry1 (Fig. 2a); at ZT6, ZT10 and ZT18 for Cry2 (Fig. 2b); at ZT6 for Rev-Erb α (Fig. 2c); at ZT22 for Ror γ (Fig. 2d). The effect of CR on the expression of these genes was also affected by the sex. The expression of the Cry1 gene was induced by CR at several time points (ZT14-ZT2) in males but CR did not have any effect on the expression of Cry1 in females (Fig. 2a). By contrast, the expression of Cry2 was not significantly affected by CR neither in males, in agreement with our previous work, nor in females (Fig. 2b). It is worth noting, according to the rhythmic analysis, that the expression of Cry2 in males was rhythmic under AL and arrhythmic upon CR (Supplementary Table S2). Rev-Erb α expression was induced by CR in males only at one time point (ZT6), while in females significant induction was detected at three time points ZT2, ZT6, ZT10 (Fig. 2c). A similar pattern of response to CR was observed in the expression of Ror γ gene (Fig. 2d).

The expression of Cry1, Cry2 and Rev-Erb α and Ror γ was significantly affected by the sex of the mice. In the AL group the expression of all four genes was significantly higher in the liver of female mice: at ZT18, ZT22 and ZT2 for Cry1 (Fig. 2a); at ZT6, ZT10 and ZT18 for Cry2 (Fig. 2b); at ZT6 for Rev-Erb α (Fig. 2c); at ZT22 for Ror γ (Fig. 2d). The effect of CR on the expression of these genes was also affected by the sex. The expression of the Cry1 gene was induced by CR at several time points (ZT14-ZT2) in males but CR did not have any effect on the expression of Cry1 in females (Fig. 2a). By contrast, the expression of Cry2 was not significantly affected by CR neither in males, in agreement with our previous work, nor in females (Fig. 2b). It is worth noting, according to the rhythmic analysis, that the expression of Cry2 in males was rhythmic under AL and arrhythmic upon CR (Supplementary Table S2). Rev-Erb α expression was induced by CR in males only at one time point (ZT6), while in females significant induction was detected at three time points ZT2, ZT6, ZT10 (Fig. 2c). A similar pattern of response to CR was observed in the expression of Ror γ gene (Fig. 2d). Ror γ expression was induced by CR in males, again, at only one time point (ZT22) while in females significant induction was detected at three time points: ZT6, ZT14, ZT18 (Fig. 2d). Thus, out of four clock genes, expressions of which were showing sexual dimorphism in the liver, CR affected the expression of one (Cry1) only in males; CR affected the expression of two (Rev-Erb α, Ror γ) in both sexes, but in females the induction was stronger and observed at several time points; finally, CR did not affect the expression of one of these genes (Cry2) in either sex.

Effect of sex on the circadian rhythms in the expression of CR regulated genes. Several genes have been identified through previous studies as targets of CR and proposed as longevity candidate genes [21]. We have found that in the liver their expression is regulated exclusively by CR but not by time restricted feeding or fasting. It was also reported that the expression of some of them is sexually dimorphic. We analyzed the expression of Fmo3, Serpina12, Mup4, Cyp4a12b and Cyp4a14a. The results are presented in Figs 3 and 4. The expressions of all these genes showed sexual dimorphism: Fmo3, Mup4 and Cyp4a12b expression was different between sexes at all time points throughout the day; Serpina12 expression was different between males and females at ZT2, ZT10-14 (Fig. 4b) and Cyp4a14a expression differed only at ZT6 (Fig. 3b). Under AL conditions Fmo3 expression was 20–100 fold (depending on the time of the day) higher in females than in males (Fig. 3a). These results are in agreement with the previously reported sexual dimorphism of Fmo3 expression [22, 23]. Fmo3 expression was induced by CR in both males and females, again in agreement with the previously published data [24]. There was a dramatic difference between sexes in the magnitude of the CR effects on the expression. In females, Fmo3
Figure 3. Sexual dimorphism and sex independent expression and response to CR for genes upregulated by CR. The daily rhythms in expression of mRNA for Fmo3 (a) and Cyp4a14a (b) in the liver: blue diamonds and dashed lines – AL male mice; red squares and solid lines – CR male mice, green triangles and dashed lines – AL female mice; purple x and solid lines – CR female mice. For all panels graphs are double plotted. Light is on at ZT0 and off at ZT12. a, b, c, d – statistically significant difference (p < 0.05) between indicated groups at time point. Open bars represent light and black bars represent dark phase of the day. Food for CR group was provided at ZT14. At every time of the day 3 mice of each sex were used for each diet.

Figure 4. Sexual dimorphism and sex independent expression and response to CR for genes downregulated by CR. The daily rhythms in expression of mRNA for Mup4 (a), Serpina12 (b) and Cyp4a12b (c) in the liver: blue diamonds and dashed lines – AL male mice; red squares and solid lines – CR male mice, green triangles and dashed lines – AL female mice; purple x and solid lines – CR female mice. For all panels graphs are double plotted. Light is on at ZT0 and off at ZT12. a, b, c, d – statistically significant difference (p < 0.05) between indicated groups at time point. Open bars represent light and black bars represent dark phase of the day. Food for CR group was provided at ZT14. At every time of the day 3 mice of each sex were used for each diet.
expression was induced 2–5 fold, and in males the expression was induced by 600–3000 fold. Due to this difference in the response to CR, under CR conditions
\( Fmo3 \) expression was significantly higher in males, while under AL control conditions the expression was higher in females.

The expression of \( Serpina12, Mup4 \) and \( Cyp4a12b \) under AL conditions was several fold higher in males and it was significantly reduced upon CR in males only. The tendency toward reduced expression was observed in females, but the basal level was low and CR did not have a statistically significant effect (Fig. 4). There was no difference in the expression of \( Cyp4a14a \) gene between males and females at 5 time points under AL conditions, and the expression was higher in females only at ZT6 (Fig. 3b). CR induced the expression in males at all time points throughout the day, however in females the induction was observed only at ZT14 time point and CR rather caused a phase shift in expression pattern of \( Cyp4a14a \) in females.

Thus, out of 5 tested genes four: \( Fmo3, Serpina12, Mup4 \) and \( Cyp4a12b \) genes have strong sexual dimorphism in expression and for \( Cyp4a14a \) gene the dimorphism has only been observed at a single time point.

Effect of sex on the circadian rhythms in protein expression. To assay whether the observed sexual dimorphism in mRNA expression translates into dimorphism in protein expression, we analyzed the expression of several genes by western blotting. The results are presented on Fig. 5. In agreement with mRNA data the expression of \( CRY1 \) protein was significantly higher in female livers under AL and CR conditions. Under AL and CR conditions the sex-based difference in protein levels of \( CRY1 \) was similar to the difference observed between sexes on mRNA level – females showed higher protein expression at ZT18-22 under AL and ZT2-6; ZT18-22 under CR (Fig. 5a,b). CR induced the expression in males at two time points (Fig. 5d). Under AL conditions the expression of \( FMO3 \) protein was several fold higher in female livers than in male at all 6 time points throughout the day (Fig. 5e), again in agreement with the mRNA data. The difference between sexes was not significant for CR samples except for ZT22. (Fig. 5f) Thus, we observed some correlation between mRNA and protein data on sexual dimorphism in the expression of circadian clock and longevity candidate genes. At the same time, an absolute correlation between mRNA and protein data is relatively
rare, the protein expression is regulated on multiple levels: translational and posttranslational. The circadian clock is involved in the posttranscriptional control of gene expression on multiple levels.

**Effect of sex on circadian rhythms in Igf-1 expression.** To extend our observations on sexual dimorphism in circadian rhythms and responses to CR we decided to investigate a signaling pathway relevant to CR, circadian clock and aging. We recently reported that Cryptochromes are involved in the regulation of Insulin like growth factor 1 (Igf-1) expression. We assayed the liver expression of Igf-1 gene in male and female mice on both diets. The results are presented in Fig. 6. We found that Igf-1 mRNA expression is higher in females than in males under AL conditions. For both sexes, CR caused the reduction in the expression, but the expression in females still remained higher (Fig. 6). Thus, the expression of Igf-1 demonstrated sexual dimorphism at both diets.

**Discussion**

The role of circadian clocks and rhythms in physiology and metabolism are well recognized. The connection between the peripheral circadian clocks and aging is also established. Clock disruption through genetic ablation of clock genes: Bmal1 and Clock results in reduced lifespan in mice14, 25, 26 and Period 2 ablation yields mixed outcomes: under regular conditions mutant mice live longer than wild type17 but after whole-body irradiation their lifespan is shorter than that of wild type mice14. Clock disruption leads to reduced lifespan in the Drosophila model1, which suggests the conservation of clock mechanisms in aging. Recent data also support the hypothesis that circadian clocks are a part of CR-modulated mechanisms. In both Drosophila8 and mouse models, CR significantly affects the rhythms in clock gene expression in peripheral organs, causing the change in the amplitude of the oscillations and the absolute levels of expression. Lifespan extension induced by CR is significantly compromised in flies with clock disruption8. In mammals the effect is even more dramatic, CR fails to increase the lifespan of mice deficient for Bmal125. These data together suggest a close interaction between the circadian clocks, CR and aging.

There is more and more evidence that sex strongly influences many physiological processes. Circadian rhythms in physiology and behavior are influenced by sex2, 18, 20. Indeed, there is a sex difference in SCN morphology and signaling to and from SCN10, which might contribute to the sex difference in many of the central clock controlled processes such as the timing of activity10. Sex may also affect the peripheral clocks and the entrainment of SCN independent circadian rhythms such as food anticipatory activity10, 17.

Many beneficial effects of CR, including the lifespan increase, are significantly affected by the sex. For example, for C57B/6J mice (the same strain that was used in our study), 20% CR increases lifespan in females more significantly than in males, while 40% CR increases lifespan in males but not in females5. Effects of CR on changes in body temperature, changes in glucose and insulin levels, changes in liver metabolism and gene expression were strongly influenced by the sex of the mice1.

Here we compared the effect of CR on the expression of circadian clock genes in the liver between male and female mice on AL and 30% CR diets. We did not find any sexual dimorphism in the expression or response to CR for circadian clock genes Bmal1, Per1, Per2 and Per3. The expression of several clock genes: Cry1, Cry2, Rev-Erb
α and Ror γγ was significantly different between males and females on both diets used. In addition, the effect of CR on the expression of Cry1, Rev-Erb α and Ror γ was sex-dependent.

There are only a few reports on the effects of sex on clock gene expression. A different phase in the expression of Per2 gene between males and females has been reported for pituitary29. Sexual dimorphism in the response to experimental chronic jet lag was observed for several clock genes: Clock, Cry2, Rev-Erb α and Bmal1; no sexual dimorphism was observed for Per1 or Per2 genes30. To the best of our knowledge, there have been no studies investigating the effect of sex on Cry1 or Ror γ expression in circadian manner. What could be the physiological significance of this difference in clock gene expression and response to CR? The sexual dimorphism in liver gene expression depends on transcriptional factor STAT5b. Recently CRYs have been implicated in the regulation of STAT5b activity29 and it was also previously reported that CRYs are essential for sexual dimorphism in liver gene expression30. Thus, the sexual dimorphism here observed in Cry gene expression might be a contributing factor, which warrants further study on the interaction between diet, CRYs and sexual dimorphism.

Sex also affected circadian rhythms in the expression of several genes regulated by CR. Flavin-containing monoxygenase 3 (FMO3) is responsible for the oxidation of trimethylamine (TMA) to trimethylamine-N-oxide (TMAO)31. Fmo3 is known to be one of the genes most strongly induced by CR in liver, and it was reported that Fmo3 homolog is involved in the determination of lifespan in nematodes32. It is also known that Fmo3 expression is strongly affected by sex in mice, the expression is high in females and practically undetectable in males33. In accordance with previously published data, we found that Fmo3 expression was higher in females than in males under AL conditions and that CR induced the expression in both sexes but with a different magnitude. Serpina12 gene encodes a serine protease inhibitor from the serpin superfamily. The protein is known as vaspin and it is implicated in the control of metabolism, insulin sensitivity and glucose homeostasis34. The level of vaspin in human blood serum was found to be oscillating in circadian manner35 and sexual dimorphism in circulating vaspin levels in obese individuals was also reported35. To the best of our knowledge sexual dimorphism in mRNA expression and the dimorphism in response to CR for Serpina12 gene have not been previously reported. Map4 gene encodes major urinary protein 4, which belongs to the family of male-specific proteins that can bind odorant molecules and regulate their release from the scent marks36. The observed sexual dimorphism in the expression of Map4 was expected and male-specific response to CR was expected as well36. Cyp4a12b and Cyp4a14a genes encode the enzymes that belong to the cytochromeP450 superfamily. These enzymes are involved in omega-hydroxylation of fatty acids and are implicated in the regulation of blood pressure37, 38. Cyp4a12b demonstrated a strong male-specific expression (Fig. 4) throughout the day, which is in agreement with reports by Jeffery et al.39 and Zhang et al.40. It was not reported previously that the effect of CR on the expression of members of the Cyp4a family is sex dependent. Igf-1 is an evolutionary conserved regulator of longevity. It is well documented that the Igf-1 expression is reduced in response to CR and might be different between males and females. The sexual dimorphism in the expression of Igf-1 mRNA for both diets reported here might contribute to the difference in longevity and the effects of CR between sexes.

The expressions of some genes showing sexual dimorphism (Fmo3, Map4 and Cyp4a12) are regulated by gonadal hormones. It is unknown if the expression of circadian clock genes might be regulated by gonadal hormones. Can the observed sexual dimorphism be a simple consequence of the difference in gonadal hormone levels? Several facts contest this simplistic explanation. In agreement with the model of the “feminizing” effect of CR on liver gene Cry1 expression was induced by CR only in males and Fmo3 was induced much stronger in males than in females. However, under CR diet the expression of Fmo3 in the liver of males is tenfold higher than in females; the expression of Cry2 was affected by CR only in females and the expression of Rev-Erb α and Ror γ was induced in both sexes. Plus, the CR induced reduction in testosterone levels in males was only about 11%41, so testosterone levels were still significantly higher in males than in females. These data suggest the existence of a more complex mechanism of regulation, not just a simple response to changes in gonadal hormone levels.

In summary, there is more and more evidence that sex is an important variable in many physiology functions not necessarily related exclusively to reproduction. Our data on the sexual dimorphism in clock gene expression and sexual dimorphism in CR-induced effects support this opinion. There is some controversy on the benefits of CR, multiple factors might influence the response of the organism to CR and present data suggest that the interaction between sex and circadian rhythms must be taken into consideration for optimal CR implementation.

Materials and Methods

Ethics Statement. All the animal studies were performed with approval from the Institutional Animal Care and Use Committee (IACUC) of Cleveland State University (Protocol No. 21124-KON-S). The care and use of mice were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of the Cleveland State University.

Experimental animals. Wild type mice were of C57B6J background. Mice were maintained on the 12:12 light:dark cycle with lights on at 7:00 am and off at 7:00 pm and fed the 18% protein rodent diet (Harlan). The ad libitum (AL) group had unrestricted access to food. Calorie restriction (CR) was started at 3 months of age. For the first week of the diet the animals were on 10% restriction, for the second week – on 20% restriction and on 30% restriction for the rest of their life and subsequent experimental measurements. The CR groups received their food once per day at ZT14 (two hours after light off). After two months of CR the tissues were collected for analysis. For AL groups the tissues were collected for 5-month-old (age match) animals. For all groups the tissues were collected at six different time points throughout the day. All groups had unrestricted access to water.

RNA isolation and analysis of mRNA expression. For gene expression studies, tissues were collected every four hours throughout the day and stored at –80°C. Total RNA was isolated from the liver using TriZol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. Briefly, frozen liver piece was minced in 1 ml TriZol reagent with pestle on ice. Following chloroform extraction step, total RNA was precipitated with isopropanol.
by centrifugation. The RNA pellet was dissolved in water and quantified on Nanodrop, RNA quality was checked on 1% agarose gel. The RT mix was prepared using 1 µg of total RNA, 50 ng of random hexamer (N808127, Invitrogen), 10 mM dNTP (DD9085, Biobasic), 0.1 M DDT and RNaseOUT Recombinant RNase inhibitor (10777-019, 40 units/µl) and it was reverse transcribed using SuperScript III Reverse Transcriptase (18080-044, Invitrogen). RNA quantification was performed using qPCR with Universal SYBR Green Mix (1725125, BioRad). The reaction was carried out in triplicates for the gene of interest and in duplicates for the normalizing control using CFX96 Real-Time PCR Detection System (BioRad) with 50ng of cDNA. Thermal cycling conditions used were according to the instruction of SYBR Green mix protocol and relative mRNA abundance was calculated using the comparative delta-Ct method with ribosomal 18S rRNA as reference gene as described in44. Water was used as negative control for qPCR analysis. Product specificity was confirmed by melting curve analysis while primer pair efficiency was calculated by generating standard curve using serial dilutions of standard. Primers used for the analysis of expression are listed in the Supplementary Table S1.

Immunoblot analysis. For analysis of protein expression tissues from three mice per every time point were used for both sexes and each feeding regimen. Figure 5 presents the quantification of protein expression from western blot experiments in which three liver samples from individual mice (N = 3) were run to estimate a variability between biological replicates and calculate means and errors. The images presented in Supplementary Figure S1 and S2 demonstrate the respective protein levels in liver samples pooled together from three different mice per each time point for each feeding regimen and sex. For the preparation of lysates, frozen liver pieces were lysed in cell signaling lysis buffer with Protease/Phosphatase Inhibitor Cocktail (Cell Signaling Technology, Beverly, MA, USA) using sonicator. Protein concentration was determined by Bradford protein assay kit according to manufacturer’s protocol using spectrophotometer and lysates were stored at –80C. 45ug of protein was loaded on 3–8% tris-acetate and 4–12% bis-tris gels (Invitrogen). Protein was transferred on PVDF membrane at 110 mAmp. Equal loading of proteins was checked by Ponceau stain. Primary antibodies: rabbit-raised polyclonal anti-CRY1 (Signalway Antibody, 21414); rabbit-raised polyclonal anti-CRY2 (Santa Cruz Biotechnology, sc-130731); rabbit-raised monoclonal anti-EMO3 (Abcam, ab126711), mouse-raised monoclonal anti-β-ACTIN (Sigma Aldrich, A5441) were used for Immunoblot analyses. All of the primary antibodies were diluted in 5% BSA in proportions indicated in protocols by the respective manufacturers. Secondary antibodies used: Anti-rabbit IgG, HRP linked (Cell Signalling, 7074 S) and Anti-mouse IgG, HRP linked (Cell Signalling, 7076S). Blot images were made using Scientific Imaging film and Odyssey FC imaging system (LI-COR). Protein analysis and quantification was done using Image Studio Lite Version 5.2 software.

Statistical analysis. For all experiments, at least three male or female mice for every time point and for each feeding type were used (N = 3). Data are shown as average +/- standard error of mean. IBM SPSS Statistics 20 and GraphPad Prism Version 5.04 software packages were used for statistical analysis. To assay the effects of sex, diet, and the time of the day, the analysis was performed using two-way repeated ANOVA. If the effect of feeding, time or sex was found to be statistically significant Bonferroni correction was used to calculate p-value for pairwise comparison. P < 0.05 was considered as a statistically significant difference. For the analysis of Circadian Rhythms in gene expression “R” Version 3.2.5 software – Cosinor Analysis package was used and the results are summarized in the Supplementary Tables S2 and S3.

References
1. Froy, O. & Miskin, R. Effect of feeding regimens on circadian rhythms: Implications for aging and longevity. Aging (Albany NY). 2, 7–27 (2010).
2. Mitchell, S. et al. Effects of Sex, Strain, and Energy Intake on Hallmarks of Aging in Mice. Cell Metab. 23, 1093–112 (2016).
3. Patel, S. A., Chaudhari, A., Gupta, R., Velingkaar, N. & Kondratov, R. V. Circadian clocks govern calorie restriction-mediated life span extension through BMAL1- and IGF-1-dependent mechanisms. FASEB J. 30, 1634–1642 (2016).
4. Nakahata, Y. et al. The NAD+–Dependent Deacetylase SIRT1 Modulates CLOCK-Mediated Chromatin Remodeling and Circadian Control. Cell 134, 329–340 (2008).
5. Khapre, R. V. et al. BMAL1-dependent regulation of the mTOR signaling pathway delays aging. Aging (Albany NY). 6, 48–57 (2014).
6. Kondratov, R. V. & Kondratova, A. A. Rapamycin in preventive (very low) doses. Aging (Albany NY). 6, 158–159 (2014).
7. Patel, S. A., Velingkaar, N., Makwana, K., Chaudhari, A. & Kondratov, R. Calorie restriction regulates circadian clock gene expression through BMAL1 dependent and independent mechanisms. Sci. Rep. 6, 25970 (2016).
8. Katewa, S. D. et al. Peripheral Circadian Clocks Mediate Dietary Restriction-Dependent Changes in Lifespan and Fat Metabolism in Drosophila. Cell Metab. 23, 143–154 (2016).
9. Green, C., Takahashi, I. & Bass, J. The Meter of Metabolism. Cell 144, 724–732 (2008).
10. Lowery, P. L. & Takahashi, J. S. MAMMALIAN CIRCADIAN BIOLOGY: Eliciting Genome-Wide Levels of Temporal Organization. Annu. Rev. Genomics Hum. Genet. 5, 407–41 (2004).
11. Potter, D. G. M. et al. Circadian Rhythm and Sleep Disruption: Causes, Metabolic Consequences and Countermeasures. Endocr. Rev. 37, e2016–1083 (2016).
12. Touitou, Y., Kopchick, J. & Bass, J. The Meter of Metabolism. Cell 144, 724–732 (2008).
13. Sahar, S. & Sassone-Corsi, P. Regulation of Metabolism: The Circadian Clock dictates the Time. Trends Endocrinol. Metab. 23, 1–8 (2012).
14. Fu, L., Pelciano, H., Liu, J., Huang, P. & Lee, C. C. The circadian gene Period2 plays an important role in tumor suppression and DNA damage response in vivo. Cell 111, 41–50 (2002).
15. Reppert, S. & Weaver, D. Coordination of circadian timing in mammals. Nature 418, 935–941 (2002).
16. Sato, T. K. et al. Feedback repression is required for mammalian circadian clock function. Nat. Genet. 38, 312–319 (2006).
17. Oosterman, J. E., Kalsbeek, A., La Fleur, S. E. & Belsham, D. D. Impact of nutrients on circadian rhythmicity. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 308, R337–R350 (2015).
18. Kira, J. A. & Mintz, E. M. Sex differences in behavioral circadian rhythms in laboratory rodents. Front. Endocrinol. (Lausanne). 5, 1–6 (2014).
19. Yan, L. & Silver, R. Neuroendocrine underpinnings of sex differences in circadian timing systems. J. Steroid Biochem. Mol. Biol. 160, 118–26 (2016).
20. Hadden, H., Soldin, S. J. & Massaro, D. Circadian disruption alters mouse lung clock gene expression and lung mechanics. J. Appl. Physiol 113, 385–392 (2012).
21. Swindell, W. R. Gene expression profiling of long-lived dwarf mice: longevity-associated genes and relationships with diet, gender and aging. BMC Genomics 8, 353 (2007).

22. Falls, J., Ryu, D., Cao, Y., Levi, P. & Hodgson, E. Regulation of mouse liver flavin-containing monoxygenases 1 and 3 by sex steroids. Arch. Biochem. Biophys. 342, 212–23 (1997).

23. Ripp, S., Itagaki, K., Phulpot, R. & Ellarida, A. Species and sex differences in expression of flavin-containing monoxygenase form 3 in liver and kidney microsomes. Drug Metab. Dispos. 27, 46–52 (1999).

24. Fu, Z. D. & Klaassen, C. D. Short-term Calorie Restriction Feminizes the mRNA Profiles of Drug Metabolizing Enzymes and Transporters in Livers of Mice. Toxicol. Appl. Pharmacol. 274, 137–146 (2014).

25. Kondratov, R. V et al. Early aging and age-related pathologies in mice deficient in BMAL1, the core component of the circadian clock service Early aging and age-related pathologies in mice deficient in BMAL1, the core component of the circadian clock. 1868–1873 doi:10.1101/gad.1832206 (2006).

26. Dubrovsky, Y. V., Samsa, W. E. & Kondratov, R. V. Deficiency of circadian protein CLOCK reduces lifespan and increases age-related cataract development in mice. Aging (Albany NY). 2, 936–944 (2010).

27. Wang, J. et al. Per2 induction limits lymphoid-biased haematopoietic stem cells and lymphopoiesis in the context of DNA damage and aging. Nat. Cell Biol. 18, 480–490 (2016).

28. Kuljis, D. et al. Gonadal- and Sex-Chromosome-Dependent Sex Differences in the Circadian System. Endocrinology 154, 1501–1512 (2013).

29. Chaushadi, A., Gupta, R., Patel, S., Velingkaar, N. & Kondratov, R. V. Cryptochromes regulate IGF-1 production and signaling through control of JAK2-dependent STAT5B phosphorylation. Mol. Biol. Cell 28, 834–842 (2017).

30. Bur, I. M. et al. The circadian clock components CR1Y and CR2 are necessary to sustain sex dimorphism in mouse liver metabolism. J. Biol. Chem. 284, 9066–9073 (2009).

31. Shih, D. M. et al. Flavin containing monoxygenase 3 exerts broad effects on glucose and lipid metabolism and atherosclerosis. J. Lipid Res. 56, 22–37 (2015).

32. Leiser, S. F. et al. Cell Non-Autonomous Activation of Flavin-containing Monoxygenase Promotes Longevity and Healthspan. Science (80-.). 350, 1375–1378 (2015).

33. Fu, Z. D., Csanaky, I. L. & Klaassen, C. D. Effects of aging on mRNA profiles for drug-metabolizing enzymes and transporters in livers of male and female mice. Drug Metab. Dispos. 40, 1216–1225 (2012).

34. Dimova, R. & Tankova, T. The role of vaspin in the development of metabolic and glucose tolerance disorders and atherosclerosis. Biomed Res. Int. 2015, (2015).

35. Jeong, E. et al. Circadian rhythm of serum vaspin in healthy male volunteers: relation to meals. J. Clin. Endocrinol. Metab. 95, 1869–75 (2010).

36. Moradi, S., Mirzaei, K., Abdurahman, A., Keshavarz, S. & Hossein-Nezhad, A. Mediatory effect of circulating vaspin on resting metabolic rate in obese individuals. Eur. J. Nutr. 55, 1297–305 (2016).

37. Sharrow, S. D., Vaughn, J. L., Żidek, L., Novotny, M. V & Stone, M. J. Pheromone binding by polymorphic mouse major urinary proteins. 2247–2256 doi:10.1110/ps.0204202.fects (2002).

38. Giller, K., Huebbe, P., Doering, F., Pallauf, K. & Rimbach, G. Major urinary protein 5, a scent communication protein, is regulated by dietary restriction and subsequent re-feeding in mice. Proceedings. Biol. Sci. 280, 20130101 (2013).

39. Muller, D. N. et al. Mouse Cyp4a isoforms: enzymatic properties, gender- and strain-specific expression, and role in renal 20-hydroxyeicosatetraenoic acid formation. Biochem. J. 403, 109–18 (2007).

40. Okita, R. & Okita, J. Cytochrome P450 4A fatty acid omega hydroxylases. Curr. Drug Metab. 2, 265–81 (2001).

41. Jeffery, B. et al. Peroxisome proliferator activated receptor α regulates a male-specific cytochrome P450 in mouse liver. Arch. Biochem. Biophys. 429, 231–236 (2004).

42. Zhang, Y. & Klaassen, C. D. Hormonal regulation of Cyp4a isoforms in mouse liver and kidney. Xenobiotica. 43, 1055–63 (2013).

43. Müller, M. J. et al. Metabolic adaptation to caloric restriction and subsequent refeeding: The Minnesota Starvation Experiment revisited. Am. J. Clin. Nutr. 102, 807–819 (2015).

44. Khapre, R. V. et al. Metabolic clock generates nutrient anticipation rhythms in mTOR signaling. Aging (Albany NY). 6, 675–689 (2014).

Acknowledgements

This work was supported by the National Institute of Health (NIH) grant (R01AG039547 to RVK) and funds from the Center of Gene Regulation in Heath and Disease (GRHD) CSU to RVK and Dissertation Research Award (DRA) from CSU to SP.

Author Contributions

A.A. and R.K. designed the research. A.A. and S.P. did the experiments and performed statistical analysis. A.A. and R.K. interpreted data and wrote the manuscript. All authors reviewed and commented on the manuscript.

Additional Information

Supplementary information accompanies this paper at doi:10.1038/s41598-017-09289-9

Competing Interests: The authors declare that they have no competing interests.

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