Time-dependent regulation of hepatic cytochrome P450 mRNA in male liver-specific PGC-1 knockout mice

Sundekilde, Ulrik Kræmer; Kristensen, Caroline Maag; Olsen, Mette Algot; Pilegaard, Henriette; Rasmussen, Martin Krøyer

Published in:
Toxicology

DOI:
10.1016/j.tox.2022.153121

Publication date:
2022

Document version
Publisher's PDF, also known as Version of record

Document license:
CC BY

Citation for published version (APA):
Sundekilde, U. K., Kristensen, C. M., Olsen, M. A., Pilegaard, H., & Rasmussen, M. K. (2022). Time-dependent regulation of hepatic cytochrome P450 mRNA in male liver-specific PGC-1 knockout mice. Toxicology, 469, [153121]. https://doi.org/10.1016/j.tox.2022.153121
Time-dependent regulation of hepatic cytochrome P450 mRNA in male liver-specific PGC-1α knockout mice

Ulrik Kræmer Sundekilde a, Caroline Maag Kristensen b, Mette Algot Olsen b, Henriette Pilegaard b, Martin Kroýer Rasmussen b,*

a Department of Food Science, Aarhus University, Denmark
b Department of Biology, University of Copenhagen, Denmark

1. Introduction

Numerous biological events display circadian patterns, e.g. wake-sleep cycles and feeding behavior. Moreover, several physiological events, like blood pressure and hormone secretion also exhibit circadian rhythms, resulting in fluctuations in energy metabolism. The circadian rhythm output has its origin in the master clock, located in the suprachiasmatic nuclei of the anterior hypothalamus, which are ultimately synchronized by the light-dark event (Reppert and Weaver, 2002). Moreover, local clock also regulates circadian rhythm in specific organs. Thus, Bmal1 (brain and muscle ARNT-like1) and CLOCK (circadian lomomotor output cycle kapa1) are essential transcription factors in the local circadian regulation (Panda et al., 2002; Reppert and Weaver, 2002). Bmal1 and CLOCK provide a positive pillar of the circadian oscillation, while CRY (cryptochromes) and PER (periods) act as negative pillars (Froy, 2009; Lu et al., 2020). This indicates that circadian fluctuations are mainly caused by events at the transcriptional level.

Among an ever evolving list of events, circadian rhythms have been shown to influence drug efficacy by influencing absorption, distribution, metabolism and excretion (Musiek and Fitzgerald, 2013). For example it has been shown that treatment of colon rectal cancer in mice was more efficient when drugs were administrated 7 h after light onset than 15 h after light onset (Granda et al., 2002). Although it was not assessed in the study, the difference in efficacy of treatment might be explained by circadian variation in hepatic drug metabolism. The hepatic metabolism of drugs are generally conducted in two phases carried out by distinct groups of enzymes. For phase I, the hepatic cytochrome p450’s (Cyp) are the main enzyme-group. Accordingly, Cyps, including Cyp2a5, Cyp2b10, Cyp2e1 and Cyp3a11 have been shown to display circadian
The transcriptional regulation of the Cyps is orchestrated by a number of different transcription factors. The bHLH/PAS (basic helix-loop-helix-Per-ARNT-sim) family member, aryl hydrocarbon receptor (AhR) is the main regulator of Cyp1-family transcription, while the clock family 1, 2, 3 and 4 was determined in male liver-specific PGC-1α knockout (LKO; n = 10), and the other half were littermate lox/lox control mice (LOX; n = 10) (for details on the mice see Kristensen et al. (2018)).

The OliGreen kit (Invitrogen) was used to estimate the content of mRNA and protein content has been documented (Zhao et al., 2019). The liver was quickly removed, immediately snap-frozen in liquid nitrogen and stored at −80 °C.

2.2. RNA extraction and real-time PCR

Total RNA from the liver samples (approx. 20 mg) was extracted using TRIreagent (Sigma-aldrich) according to the manufacturer’s instructions. The OliGreen kit (Invitrogen) was used to estimate the content of single-stranded DNA (ssDNA) in each cDNA sample as previously described (Lundby et al., 2005).

2.3. Quantification of cDNA content

The OliGreen kit (Invitrogen) was used to estimate the content of single-stranded DNA (ssDNA) in each cDNA sample as previously described (Lundby et al., 2005).
phenylmethylsulfonyl, and incubated under rotation (head-over-end) at 4 °C for 2 h. Following 20 min centrifugation at 20,000 g (4 °C), the supernatant was collected and subjected to determination of total protein content using the Pierce BCA protein kit, according to the manufacturer’s protocol (ThermoScientific). Equal amount of total protein was mixed 1:1 with Laemmli-buffer and separated using Any KD gels (Bio-Rad). Subsequently, the proteins were blotted onto PVDF membranes using the Turbo Transfer system (Bio-Rad). For this the gels were activated for 1 min prior to blotting. Membranes were blocked in TBST (50 mM Tris, 500 mM NaCl, 0.1 % Tween 20; pH 7.4) supplemented with 2% dry-milk powder for 1 h at room temperature, before incubation overnight (at 4 °C) with primary antibodies. The used antibodies were CYP1A (Santa Cruz 53241), CYP2A (Santa Cruz-53615), CYP2B (Bio-Rad, WMA00171), CYP2e1 (Abcam 28146) and CYP3A (Millipore ab1254). Following careful washing, the membranes were incubated with horseradish peroxidase conjugated secondary antibodies at room temperature for 2 h. After 3 x washing with TBST, the specific protein was visualized using ECL substrate (Bio-Rad) and the ChemiDoc-XRS workstation (Bio-Rad). Relative protein content was quantified using Image Lab (Bio-Rad) and normalized to total content of loaded protein by subtracting 1 from mRNA values >1 (indicating upregulation). The transformed data were scaled to unit variance prior to modelling. PCA was performed using Simca 16.0 (Sartorius Stedim Data Analytics, Umeå, Sweden).

### 3. Results

#### 3.1. Selection of normalization method

Four often used housekeeping genes were evaluated for their appropriateness for normalization of the target mRNA content in the present study investigating the impact of circadian rhythms on content of selected genes and lack of hepatic PGC-1α. To evaluate the stability of the expression the housekeeping-genes, two-way ANOVA was performed on the relative content (Table 2). For all the analyzed housekeeping-genes, the statistical testing showed that the mRNA level was influenced by sampling time (ZT) or genotype (only RPLP0). Moreover, for β-actin mRNA there was a significant interaction between sampling time and genotype. This suggests that traditional normalization to these housekeeping-genes can be problematic when studying circadian rhythm and hepatic PGC-1α knockout and shows that they could not be used for normalization in the present study. Therefore ssDNA was determined and used for normalization.

#### 3.2. Expression of circadian marker genes

To verify the circadian state of the mice, the hepatic mRNA content of two known circadian regulated genes was determined (Fig. 1). Bmal1 mRNA content was significantly (p < 0.001) lower at ZT-12 than at ZT-2 independent of genotype. For the mRNA content of CLOCK, there was an overall significant (p < 0.05) difference when comparing ZT-2 and ZT-12. The subsequent Tukey’s post-hoc test identified a tendency (p = 0.07) towards a difference between ZT-2 and ZT-12 only in the LOX mice.

#### 3.3. Circadian regulation of Cyp

Hepatic Cyp2a4 mRNA content was significantly (p < 0.001) higher effect was observed, Tukey’s post hoc test was used to locate differences between the groups. If equal variance test failed, data were log10 transformed before executing the ANOVA. For all tests, p < 0.05 was regarded as significant. Statistical tests were performed in SigmaPlot 11.0 (Systat Software, USA).

For principal component analysis (PCA), normalized mRNA data for 12 genes (Ahr, Car, Cyp1a2, Cyp2a4, Cyb2b10, Cyp2c49, Cyp2e1, Cyp3a11, Cyp4a10, Hnf-4a, Pparα, and Pxr) were analysed. Analysis of differentially expressed genes was enabled by reciprocal transformed of mRNA values between 0 and 1 (indicating downregulation) and by subtracting 1 from mRNA values >1 (indicating upregulation). The transformed data were scaled to unit variance prior to modelling. 

![Fig. 1. Zeitgeber time and partly hepatic PGC-1α are essential for the mRNA content of Bmal1 (A) and Clock (B) in mouse liver. Values are mean ± standard error of the mean. * Different from ZT-2 within genotype (p < .05); # tends to be different from ZT-2 within genotype (p < 0.1).](image-url)
at ZT-12 than at ZT-2 (Fig. 2) with no effect of genotype. In addition, there was significantly \( p < 0.05 \) higher hepatic Cyp2e1 mRNA content at ZT-12 than at ZT-2 and the subsequent Tukey’s post-hoc test only showed significant difference \( p < 0.05 \) in the LOX group of mice, while there was no difference \( p = 0.14 \) in the LKO group.

The mRNA content of Cyp1a2, Cyp2b10, Cyp2c29, Cyp3a11 and Cyp4a10 was not different between ZT-2 and ZT-12 (Fig. 2). Moreover there was no genotype difference in hepatic Cyp mRNA content except for Cyp2b10 mRNA, where there was an overall significant \( p < 0.05 \) difference between the LOX and LKO group (Fig. 2). However, the subsequent Tukey’s post-hoc test only showed a tendency \( p = 0.05 \) towards a difference at ZT-2, while there was no difference at ZT-12 \( p > 0.1 \).

At the protein level only Cyp2e1 displayed higher content at ZT-12 than at ZT-2, and this was restricted to the LKO mice (Fig. 3), while there was no difference in Cyp1a, Cyp2a, Cyp2b and Cyp3a protein between ZT-2 and ZT-12 (Fig. 3). On the other hand, Cyp2b protein content was lower in LKO than LOX at ZT-2 (Fig. 3).
3.4. Circadian differences in mRNA content of selected transcription factors

To further investigate the underlying mechanisms for circadian regulation of Cyp mRNA content, we determined the mRNA content of transcription factors known to regulate the investigated Cyps. Hepatic PPARα mRNA content was significantly (p < 0.01) higher at ZT-12 than at ZT-2 (Fig. 4). The subsequent Tukey’s post-hoc test only showed a significant difference (p < 0.02) in PPARα mRNA in the LKO group, while there was a tendency (p = 0.06) in the LOX group. Otherwise, the mRNA level of Ahr, Car, Pxr, and Hnf-4α did not display circadian changes in mRNA content (Fig. 4).

Overall genotype differences were observed for hepatic Car and Pxr mRNA content. This was localized to a tendency for lower hepatic Car mRNA in LKO than LOX at ZT12 and a tendency for lower hepatic Pxr mRNA in LKO than LOX at both ZT2 and ZT12 (Fig. 4).

3.5. Principal component analysis of circadian gene expression

To further analyze the potential impact of PGC-1α on the circadian expression of Cyp and related transcription factors, we performed a PCA on the mRNA data. The PCA analysis showed a clear separation of the experimental groups with ZT. (Fig. 5). Moreover, the PCA analysis further showed separation of the genotypes (Fig. 5).

4. Discussion

The main findings of the present study are that the observed circadian regulation of Cyp mRNA content in the liver was in general not dependent on the presence of hepatic PGC-1α. However, hepatic PGC-1α had a regulatory effect on the basal levels of Clock, Cyp2b10, Car, and Pxr mRNA levels in the liver.

The ZT-2 time-point was selected in order to minimize the effect of changes in physical activity and feeding due to light conditions. To verify that the two selected time-point are representative for the circadian rhythm of gene expression, we determined the mRNA content of Bmal1 and Clock as known markers of circadian rhythm. In accordance with previous studies (Liu et al., 2007; Yang et al., 2006; Zhao et al., 2019) the mRNA content of Bmal1 and Clock was lower at ZT-12 than at ZT-2. This confirms the circadian state of the mice used in the experiment. Moreover, the observation that Clock mRNA content at ZT-12 tended to be higher in the LKO group than the LOX group,
suggests that hepatic PGC-1α may have some regulatory impact on the circadian regulation of hepatic Clock transcription. It should also be noticed that it has been shown, in the same mice, that the PGC-1α mRNA content in the LOX group was 2-fold higher at ZT-12 than at ZT-2 (Kristensen et al., 2018). This observation is in accordance with previous results also obtained in liver tissue (Liu et al., 2007; Orozco-Solis et al., 2011; Sherman et al., 2011), and further verifies the circadian state of the mice.

The present observation that Cyp2a4 mRNA was higher at ZT-12 than at ZT-2 is in accordance with previous results reporting Cyp2a as well as Cyp2b’s to be differentially expressed at a diurnal rhythm (Deng et al., 2018; Lavery et al., 1999; Singh et al., 2018). In the study by Deng et al. (2018) it was demonstrated that the circadian expression of Cyp2a5 was mediated through the PPARγ. Previous studies have shown that the activity of PPARγ is partly under the control of PGC-1α (Puigserver and Spiegelman, 2003) and the present observation that PPARα mRNA and PGC-1α mRNA (Kristensen et al., 2018) were higher at ZT-12 than ZT-2 may suggest that the circadian expression pattern of cyp2a4 would be compromised in the PGC-1α LKO mice. However, the finding that the circadian regulation of Cyp2a4 was not dependent on genotype, suggests that Cyp2a4 is regulated by other mechanisms than through PGC-1α. In accordance, circadian regulation of Cyp2a4 transcription has been demonstrated to involve the PAR Leucine Zipper transcription factor DBF (albumin D-site-binding protein) (Gachon et al., 2006; Lavery et al., 1999). Moreover, the nuclear receptor Car, known to regulate the Cyp2 family, also also been shown to display circadian regulation (Daujat-Chavanieu and Gerbal-Chaloin, 2020), although circadian regulation of Car mRNA content was not observed in the present study. Interestingly, the observations that Cyp2b10 mRNA content was lower in LKO mice than LOX mice and Cyp2b protein level was lower in LKO than LOX at ZT2 indicate that PGC-1α contributes in regulating basal expression of Cyp2b10 in the liver. Although the same effect was not observed in a previous study on liver specific PGC-1α knockout mice (Thogersen et al., 2020), it has been shown in mice that ethanol-induced Cyp2b10 expression is partly dependent on PGC-1α (Koga et al., 2016). This might be caused by the ability of PGC-1α to increase ligand-independent Car activity at the Cyp2b10 promotor (Ding et al., 2006). However, this need to be addressed in future studies.

The present observation that Cyp2e1 mRNA and protein content in the LOX group was higher at ZT-12 than at ZT-2 is in accordance with
Toxicology 469 (2022) 153121
7

the previous observation that hepatic Cyp2e1 mRNA and protein content as well as activity displayed circadian rhythm in mice and rats (Ge et al., 2021; Khemawoot et al., 2007; Matsunaga et al., 2008). Moreover, the observation that the statistical time difference in Cyp2e1 mRNA content only was observed in the LOX group may suggest that hepatic presence of PGC-1α is important for the circadian regulation of this Cyp isoform. However, it should be noticed that under other conditions, e.g. fasting, hepatic PGC-1α has been shown not to have an impact on Cyp2e1 content (Thogersen et al., 2020). In fact the transcription factor Period1 has recently been shown to mediate the circadian control of Cyp2e1 transcription (Ge et al., 2021). Likewise, hepatic nuclear factor-1α has also been documented to positively control the circadian rhythm of Cyp2e1 expression through binding to the Cyp2e1 gene promoter, while Cry has been reported to negatively regulate Cyp2e1 expression (Matsunaga et al., 2008). In mice, whole body knockout of PGC-1α was shown to have a minor but significant hampering of the circadian expression of Cry and Per (Liu et al., 2007). Thus, the disruption of the regulatory axis of PGC-1α-Clock-Cry/Per-Cyp2e1 in the PGC-1α LKO mice may explain that the circadian rhythm of Cyp2e1 expression only was observed in the LOX mice in the current study. This is further supported by the effect of hepatic PGC-1α knockout on Clock mRNA content observed in the present study.

As others have observed circadian expression of hepatic Cyp3a11 in mice (Lin et al., 2019; Zhao et al., 2020) and CYP3A4 in serum-shocked HepG2 and HepaRG cell-lines (Chen et al., 2020; Takiguchi et al., 2007), we also expected to observe such effects in the liver. However, the observation that the mRNA content of Cyp3a11 and Pxr mRNA was similar at ZT-2 and ZT-12 does not support the existence of such regulation, which may be explained by the differential expression of Cyp3a11 between sexes. For example the basal content of Cyp3a11 mRNA and protein has been reported to be higher in male mice than in female mice (Chen et al., 2018; Thogersen et al., 2020). Moreover, the circadian rhythm of the Cyp3a11 mRNA content also seems to differ between sexes as larger fluctuation has been observed in female mice than in male mice (Lu et al., 2013; Singh et al., 2018). Thus, the use of male mice in the present study, may partially explain why no difference was observed in Cyp3a11 mRNA between ZT-2 and ZT-12.

The present finding that the hepatic mRNA content of the four commonly used housekeeping genes Rplp0, β-actin, Gapdh and Eif2a was similar at ZT-2 and ZT-12 does not support the existence of such regulation, which may be explained by the differential expression of Cyp3a11 between sexes. For example the basal content of Cyp3a11 mRNA and protein has been reported to be higher in male mice than in female mice (Chen et al., 2018; Thogersen et al., 2020). Moreover, the circadian rhythm of the Cyp3a11 mRNA content also seems to differ between sexes as larger fluctuation has been observed in female mice than in male mice (Lu et al., 2013; Singh et al., 2018). Thus, the use of male mice in the present study, may partially explain why no difference was observed in Cyp3a11 mRNA between ZT-2 and ZT-12.

The present finding that the hepatic mRNA content of the four commonly used housekeeping genes Rplp0, β-actin, Gapdh and Eif2a was significantly different between the experimental groups underlines the importance of carefully selecting housekeeping genes for normalization when investigating the effects of circadian rhythms and hepatic PGC-1α knockout on hepatic mRNA content. This is in accordance with previous investigations demonstrating that circadian rhythm has profound effects on the mRNA content of several categories of genes (Rijo-Ferreira and Takahashi, 2019; Zhang et al., 2014). Furthermore, previous studies have used different algorithms to rank housekeeping genes from stable to less stables in expression (Hadadi et al., 2018; Kosir et al., 2010). The results from these studies revealed that no stably expressed housekeeping gene across different circadian conditions could be identified also emphasizing that a case-to-case based evaluation of the applicability of the analyzed housekeeping genes is essential for the generation of reliable mRNA results also in circadian experiments. In addition, the knockout of specific genes also introduces a risk of affecting basic expression of commonly used housekeeping genes. Therefore ssDNA was used for normalization in the current study as previously described (Lundby et al. (2005). In conclusion, the present results indicate that hepatic PGC-1α
regulates the basal expression of selected Cyp genes in the liver and provides some effects on the circadian regulation of Cyp genes in the liver. Together this suggests that PGC-1α influences the basal hepatic capacity for detoxification and contributes in regulating the circadian variation in detoxification capacity.

Declaration of Competing Interest

The authors declare no conflict of interests.

Acknowledgment

The present study was funded by the Independent Research Fund Denmark, Medical and Health Sciences.

References

Arpiaißen, S., Jarvenpa, S.M., Manninen, A., Viitala, P., Lang, M.A., Pelkonen, O., Hakola, J., 2008. Coactivator PGC-1α regulates the fasting inducible xenobiotic-metabolizing enzyme CYP2A5 in mouse primary hepatocytes. Toxicol. Appl. Pharmacol. 223, 135–141.

Bozek, K., Relogio, A., Kielbasa, S.M., Heine, M., Dame, C., Kramer, A., Herzel, H., 2009. Circadian optimisation of irinotecan and oxaliplatin efficacy in mice. Chronobiol. Int. 26, 1701–1706.

Daujat-Chavanieu, M., Gerbal-Chaloin, S., 2020. Determination of reference genes for circadian studies in different tissues and mouse strains. BMC Mol. Biol. 21, 60.

Liu, D., Zhou, C., Chen, M., Wu, B., 2020. Circadian clock-controlled drug metabolism: implications for chronotherapeutics. Drug Metab. Dispos. 48, 395–406.

Ludvhy, C., Nordsborg, N., Kusuhara, K., Kristensen, K.M., Neufler, P.D., Pilegaard, H., 2005. Gene expression in human skeletal muscle: alternative normalization method and effect of repeated biopsies. Eur. J. Appl. Physiol. 95, 351–360.

Matsunaga, N., Beda, M., Takiguchi, T., Koyanagi, S., Ohudo, S., 2008. The molecular mechanism regulating 24-hour rhythm of CYP2E1 expression in the mouse liver. Hepatology 48, 240–251.

Musiek, E.S., Fitzgerald, G.A., 2015. Molecular clocks in pharmacology. Handb. Exp. Pharmacol. 217, 243–260.

Orozco-Solis, R., Matos, R.I., Puentes, S., Sosa, S., Gómez, R., Ballesteros, J., Ochoa, J.A., 2019. Genomics of circadian rhythms in health and disease. Genomics 115, 99–100.

Panda, S., Hogenesch, J.B., Kay, S.A., 2002. Circadian rhythms from flies to humans. Nature 417, 329–335.

Rasmusson, M.K., Walsworth, R.L., 2006. Rhythmic CLOCK-BMAL1 binding to multiple E-box motifs drives circadian Dbp transcription and chromatin transitions. Nat. Genet. 38, 941–946.

Singh, M., Bergmann, L., Lang, A., Pexa, K., Kuck, F., Stibane, D., Janke, L., Ezzahoini, H., Muller, P., Petlenkof, T., Brand, R., Jochum, C., 2008. Coactivator PGC-1alpha regulates the fasting inducible hepatic CYP2E1 and its effect on disposition kinetics of chloroxzone in rats. Eur. J. Pharmacol. 574, 71–76.

Koga, T., Yama, M., Ohsawa, T., Tanimoto, Y., Nakamura, K., Nagai, Y., Kikuchi, H., 2004. Circadian regulation of perinatal nutrient restriction induces long-term changes in hepatic drug metabolism in mice. J. Biol. Chem. 279, 46455–46462.

Kosnik, R., Acimovic, J., Golicnik, M., Perse, M., Majdic, G., Fink, M., Rozman, D., 2010. Determination of reference genes for circadian studies in different tissues and mouse strains. BMC Mol. Biol. 11, 60.

Krisitensen, C.M., Olsen, M.A., Jensen, H., Brandt, N., Melgård, J.N., Pilegaard, H., 2018. PGC-1α in exercise and fasting-induced regulation of hepatic UPR in mice. Pflügers Archiv – Eur. J. Physiol. 470, 1431–1447.

Lavery, D.J., Lopez-Molina, L., Marugueno, R., Fleuray-Olela, F., Conquet, F., Schibler, U., Bonfils, C., 1999. Circadian expression of the 15 alpha hydroxylation of (5α24α) and coumarin 7-hydroxylation (Cyp2a5) genes in mouse liver is regulated by the PAR1 signaling Nrf2 transcription factor DBF, Mol. Cell. Biol. 19, 6488–6499.

Lin, Y., Xu, L., Zhao, S., Zhou, G., Wu, F., Wu, B., 2019. Bmal1 regulates circadian expression of cytochrome P450 3a1 and drug metabolism in mice. Commun. Biol. 2, 278.

Liu, C., Li, S., Liu, T., Borjigin, J., Lin, J.D., 2007. Transcriptional coactivator PGC-1α regulates the mammalian clock and energy metabolism. Nature 447, 477–481.

Liu, Y.F., Lin, X., Zhang, D., Wu, Q., Zhang, Y.K., Liu, J., 2013. Sex differences in the circadian variation of cytochrome p450 genes and corresponding nuclear receptors in mouse liver. Chronobiol. Int. 30, 1135–1143.

Lu, D., Zhao, M., Chen, M., Wu, B., 2020. Circadian clock-controlled drug metabolism: implications for chronotherapeutics. Drug Metab. Dispos. 48, 395–406.

Lundby, C., Nordsborg, J., Kusuhara, K., Kristensen, K.M., Neufler, P.D., Pilegaard, H., 2005. Gene expression in human skeletal muscle: alternative normalization method and effect of repeated biopsies. Eur. J. Appl. Physiol. 95, 351–360.

Matsunaga, N., Beda, M., Takiguchi, T., Koyanagi, S., Ohudo, S., 2008. The molecular mechanism regulating 24-hour rhythm of CYP2E1 expression in the mouse liver.

Hepatology 48, 240–251.

Musiek, E.S., Fitzgerald, G.A., 2013. Molecular clocks in pharmacology. Handb. Exp. Pharmacol. 217, 243–260.

Oladimeji, P., Cui, H., Zhang, C., Chen, T., 2016. Regulation of PXR and CAR by protein–protein interaction and signaling cross-talk. Expert Opin. Drug Metab. Toxicol. 12, 997–1010.

Orozco-Solis, R., Matos, R.I., Lopes de Souza, S., Gómez, R., Ballesteros, J., Ochoa, J.A., 2019. Circadian rhythms from flies to humans. Nature 417, 329–335.

Pascucci, J.M., Gerbal-Chaloin, S., Duret, C., Daujat-Chavanieu, M., Vilarem, M.J., Maurel, P., 2008. The role of the nuclear receptors that controls xenobiotic metabolism and transport: cross-talk and consequences. Annu. Rev. Pharmacol. Toxicol. 48, 1–32.

Puigserver, P., Spiegelman, W., 2003. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. Endocr. Rev. 24, 78–90.

Rasmusson, M.K., Zamaratskaia, G., Ekstrand, B., 2011. Gender-related differences in cytochrome P450 in porcine liver – implication for activity, expression and inhibition by testicular steriods. Reprod. Domest. Anim. 46, 616–623.

Rasmusson, M.K., Berthold, L., Gudkienė, A., Pilegaard, H., Kaur, J.G., 2019. Impact of fasting followed by short-term exposure to interleukin-6 on cytochrome P450 mRNA in mice. Toxicol. Lett. 282, 93–99.

Repetti, S.M., Weaver, D.R., 2002. Coordination of circadian timing in mammals. Nature 418, 935–941.

Rijo-Ferreira, F., Takahashi, J.S., 2019. Genomics of circadian rhythms in health and disease. Genome Med. 11, 82.

Ripperger, J.A., Schibler, U., 2006. Rhythmic CLOCK-BMAL1 binding to multiple e-box motifs drives circadian Dbp transcription and chromatin transitions. Nat. Genet. 38, 369–374.

Rius-Perez, S., Torres-Cuevas, I., Millan, I., Ortega, A.L., Perez, S., 2020. PGC-1α, peroxisome proliferator-activated receptor γ and the level of inflammatory and disease markers. J. Cell. Mol. Med. 15, 2745–2753.

Singh, M., Bergmann, L., Lang, A., Pexa, K., Kuck, F., Stibane, D., Janke, L., Ezzahoini, H., Lindecke, A., Wiek, C., Hanenberg, H., Köhrer, K., von Gall, C., Reinke, H., Piekocz, R.P., 2018. PGC-1α regulates circadian metabolic flux in murine liver. Oncotarget 9.

Takahashi, J.S., 2015. The molecular basis for circadian rhythm in liver. J. Biol. Rhythms 30, 370–378.