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

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ABSTRACT

The circadian rhythm has profound effect on the body, exerting effects on diverse events like sleep-wake patterns, eating behavior and hepatic detoxification. The cytochrome p450 s (Cyps) is the main group of enzymes responsible for detoxification. However, the underlying mechanisms behind circadian regulation of the Cyps are currently not fully clarified. Therefore, the aim of the present study was to investigate the requirement of hepatic peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) for the circadian regulation of the hepatic expression of Cyp1–4 using liver-specific PGC-1α knockout (LKO) mice and littermate controls. The circadian regulator genes Bmal1 and Clock displayed decreased mRNA content at zeitgeber time (ZT) 12, compared to ZT-2 and the mRNA content of Cyp2a4 and Cyp2e1 was higher at ZT-12 than at ZT-2. Moreover, the increase in Cyp2e1 mRNA content was not observed in the PGC-1α LKO mice and hepatic PGC-1α deficiency tended to blunt the rhythmic expression of Clock and Bmal1. However, no circadian regulation was evident at the protein level for the investigated Cyps except for a change in Cyp2e1 protein content in the LKO mice. Of the measured transcription factors, only, the mRNA content of peroxisome proliferator-activated receptor α showed rhythmic expression. To further analyze the difference between the control and LKO mice, principal component analysis were executed on the mRNA data. This demonstrated a clear separation of the experimental groups with respect to ZT and genotype. Our finding provides novel insight into the role of hepatic PGC-1α for basic and circadian expression of Cyps in mouse liver. This is important for our understanding of the molecular events behind circadian Cyp regulation and hence circadian regulation of hepatic detoxification capacity.

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 locomotor output cycle kaput) 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
oscillations at the mRNA and protein level in rodents (Deng et al., 2018; Gachon et al., 2006; Khemawoot et al., 2007; Lin et al., 2019).

The transcriptional regulation of the Cyp 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 Ahr, Aryl hydrocarbon receptor; Car, constitutive androstane receptor; Pxr, pregnane x receptor; Ppar, peroxisome proliferator-activated receptor α, Hnf-4α, hepatic nuclear factor 4α, Rplp0, ribosomal protein large P0; Gapdh, glyceraldehyde dehydrogenase; Eif2a, eukaryotic translation initiation factor 2A; Bmal1, brain and muscle ARNT-like1; Clock, circadian locomotor output cycle kaput.

Table 1

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Forward primer (5'-3')</th>
<th>Reverse primer (5'-3')</th>
<th>Probe (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyp1a2</td>
<td>TGGAGCCGCTTTGGACAGCAG</td>
<td>GCTTGGGCTTCATTGACACAG</td>
<td>CACCAACAGCAGCCTACCTGAGCATT</td>
</tr>
<tr>
<td>Cyp2b10</td>
<td>TGGAGGAGCCAGGATCCAA</td>
<td>AATGAAAGCCACCTGCTTCTCTCCT</td>
<td>AGGGGGCTTCTCTCTCATGATTCCTTCC</td>
</tr>
<tr>
<td>Cyp2c9</td>
<td>TTCTTTTTTGTGACACCACTCT</td>
<td>GGAGCCCCAGGATTGTTTGGG</td>
<td>TCCGGAGATGCTTTCGAGCCACAG</td>
</tr>
<tr>
<td>Cyp3a11</td>
<td>AACTCCGAGTAGGATGCTGAG</td>
<td>TGGCCTTAGGCTTTGTTCGAG</td>
<td>AGGGGGCTTCTCTCTCATGATTCCTTCC</td>
</tr>
<tr>
<td>Cyp3a10</td>
<td>TTCCAGTGGTCCATGAGCAGTCTC</td>
<td>AAGGCTAGGCTGCTTTCTCAGT</td>
<td>GAGGAGGCTTCTCTCTCATGATTCCTTCC</td>
</tr>
<tr>
<td>Bmal1</td>
<td>CTTAGTAGTGGTCTCGGTTGCA</td>
<td>TCAGATATTTCACCCGTATTTCC</td>
<td>GCAGAAGCCATACCTGCCACAG</td>
</tr>
<tr>
<td>Clock</td>
<td>TAGGTCGTTGTTCTCCACCCCACT</td>
<td>TGGGAGTTCCTGCTTTCTCAGT</td>
<td>AGAGAAGGCGTGTGCAG</td>
</tr>
<tr>
<td>Ahr</td>
<td>GGCGGCGCACTCAGCA</td>
<td>GCGGCGGCGAAGCTTCTTAAG</td>
<td>CGGGAAGCAATAGGGCCACAG</td>
</tr>
<tr>
<td>Car</td>
<td>TCAACAGTATTGACTGGACACA</td>
<td>TATAGGAGGCTTCAAAATAGGACTAT</td>
<td>AAGGAGGCTTCTCACTGGCCACAG</td>
</tr>
<tr>
<td>Pxr</td>
<td>CGACCTGCGGAGCTGATGCA</td>
<td>AATAGGAGGCTTCAAAATAGGACTAT</td>
<td>AAGGAGGCTTCTCACTGGCCACAG</td>
</tr>
<tr>
<td>Pparα</td>
<td>GGCGGCGAGCAATGTCG</td>
<td>GAACCTGCGGAGCAGCACAGAACACAG</td>
<td>AGGGGGCTTCTCTCTCATGATTCCTTCC</td>
</tr>
<tr>
<td>Hnf-4α</td>
<td>CTCTGCAGGTTAGCTCCAGAACA</td>
<td>AGGGCCAGGAGGAGCAGAACACAGAACACAG</td>
<td>AGGGGGCTTCTCTCTCATGATTCCTTCC</td>
</tr>
<tr>
<td>Rplp0</td>
<td>GAGCGATCCACAGAAAATCCT</td>
<td>TCTGATGCTGACAATGACTCACTCA</td>
<td>AGGCAACATGGAATATATG</td>
</tr>
<tr>
<td>α-actin</td>
<td>GCTTCTTTTGAGCTCTTTGCTCTC</td>
<td>GCGGCAACAGGATGCTTCTCCACCCACAG</td>
<td>GCGGCAACAGGATGCTTCTCCACCCACAG</td>
</tr>
<tr>
<td>Gapdh</td>
<td>GTCCAACATGATCCGCTGACCA</td>
<td>CGATGGGCGAGGTTCGTTACTCAAG</td>
<td>AAGGAGGCTTCTGCTCTGCCACAG</td>
</tr>
<tr>
<td>Eif2a</td>
<td>CTGCGAGCAGTTACGCTTCTCTCTC</td>
<td>CAGTTCGCGATGCTGTTACTCAAG</td>
<td>AAGGAGGCTTCTGCTCTGCCACAG</td>
</tr>
</tbody>
</table>

The most pronounced circadian regulation of the hepatic Cyp has been reported for Cyp2a4 and Cyp2a5. The transcriptional regulation of these genes is generally believed to be mediated by the CAR-dependent axis. Moreover, peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) has been shown to be important for the transcriptional regulation of Cyp2a5 and Cyp3a10 in primary mouse hepatocytes (Arpiainen et al., 2008) (Buler et al., 2011). Moreover, PGC-1α has been shown to regulate PXR and CAR activity via direct protein-protein interactions (Oladiji et al., 2016) and to be a key coactivator of CAR transcription factor (Gachon et al., 2006; Khemawoot et al., 2007; Lin et al., 2019).

2. Materials and methods

2.1. Animals and experimental conditions

Twenty male mice (C57/B6N) originating from a larger group of animals described in Kristensen et al. (2018), were allocated to the present study. The mice were kept on a 12:12 h’s light-dark cycle and had ad libitum access to chow and water. Half of the mice were 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)).

On the day of sampling, mice were euthanized by cervical dislocation either between 8 and 9 a.m. (2 ZT) (five LKO and four LOX (one sample was unfortunately lost) or between 5 and 6 p.m. (12 ZT) (five LKO and five LOX). 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 final pellet was air-dried and re-suspended in TE-buffer (50 mM TRIS, 1 mM EDTA, pH 8.0). RNA concentrations and purity were estimated by measuring the absorbance (MySpec, VWR) at 260 nm and the ratio 260 nm-to-280 nm, respectively. The ratio 260/280 > 1.9 for all samples. Total RNA (800 ng) from each sample was converted to cDNA using the iScript Kit (Bio-Rad), according to the manufacturer’s protocol. Specific primer and TaqMan probes (Table 1) were designed as described elsewhere (Rasmussen et al., 2018) and custom-made (LGBiosearch Technologies, Risskov, Denmark). Real-Time PCR and data handling were performed as described in Rasmussen et al. (2011).

2.3. Quantification of cDNA content

The OligoGreen kit (Invitrogen) was used to estimate the content of single-stranded DNA (ssDNA) in each cDNA sample as previously described (Lundby et al., 2005).

2.4. Western blotting

Approximately 50 μg of liver tissue were homogenized in RIPA buffer (50 mM TRIS, 150 mM NaCl, 2 mM EDTA, 1% Triton X 100, 0.5% sodium deoxycolate, 0.1 % SDS; pH 7.4) containing 2 mM
Table 2
Relative content of selected housekeeping genes in LOX and PGC-1α liver specific knockout mice obtained with Real-Time PCR.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>ZT-2 LOX</th>
<th>ZT-2 LKO</th>
<th>ZT-12 LOX</th>
<th>ZT-12 LKO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rplp0</td>
<td>±</td>
<td>±</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>β-actin</td>
<td>±</td>
<td>±</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Gapdh</td>
<td>±</td>
<td>±</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Eif2a</td>
<td>±</td>
<td>±</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard error of the mean. Rplp0, ribosomal protein large P0; Gapdh, glyceraldehyde dehydrogenase; Eif2a, eukaryotic translation initiation factor; ZT, zeitgeber time.

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 as determined using a Stain-free system (Bio-rad).

2.5. Statistical analysis

Data are presented as mean ± standard error of the mean. Two-way ANOVA was used to evaluate the effect of ZT and genotype. If an overall 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. 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

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 < .01).
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 at 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; Laverty et al., 1999). Moreover, the nuclear receptor Car, known to regulate the Cyp2 family, 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 promoter (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...
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 Perid1 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 and Pxr mRNA content 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 in the liver and provides some effects on the circadian regulation of Cyp 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

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