Research Article

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Effects of various conditions related to circadian rhythm disturbances on plasma and erythrocyte lipids in rats: a peroxisomal perspective

Sıçanlarda sirkadiyen ritim bozuklukları ile ilgili çeşitli koşulların plazma ve eritrosit lipidleri üzerindeki etkileri: peroksizomal bir bakış açısı

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Abstract

Purpose: Lipidomics studies of sleep restriction, which is known to be associated with circadian perturbations, revealed alterations in some plasma phospholipid levels including plasmalogens which are partly synthesized in liver peroxisomes. To this end it was aimed to investigate effects of various conditions known to cause circadian rhythm disturbances on various peroxisomal parameters and to compare those effects with that of fenofibrate, a peroxisome proliferator-activated receptor alpha agonist.

Materials and methods: Plasmalogens and some fatty acids in erythrocyte lysates were analyzed by GC. Peroxisomal metabolites including very long chain fatty acids as well as phytanic and pristanic acids in plasma were measured by GC-MS. Immunohistological analyses by catalase antibodies were conducted on liver sections.

Results: All the conditions tested exhibited increased catalase immunoreactivity in liver sections compared to that of controls. Both calorie restriction, time-restricted feeding, as well as fenofibrate treatment exhibited lower C18:0 plasmalogen contents of erythrocyte lysates. As plasmalogens are known to be synthesized by peroxisomes, the present results suggest that the peroxisomal lipid content in membranes might be affected by conditions co-occuring with circadian perturbations.

Conclusion: Shared effects of conditions associated with circadian rhythm disturbances and peroxisomal induction by fenofibrate on erythrocyte membrane lipids might indicate a link between them.

Keywords: Calorie restriction, circadian rhythm, fenofibrate, peroxisome, plasmalogen.

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Öz

Amaç: Lipidomik çalışmalar; sirkadiyen ritim bozukluklarıyla ilişkili olduğu bilinen uyku kısıtlamasının karaciğer peroksizomlarında sentezlenen plazmalojenler dahil olmak üzere bazı plazma fosfolipid düzeylerinde değişikliklere neden olduğunu ortaya koymuştur. Bu nedenle bu çalışmada, sirkadiyen ritim bozukluklarına neden olduğu bilinen çeşitli koşulların bazı peroksizomal parametreler üzerindeki etkilerinin araştırılması ve bu etkilerin, bir peroksizom proliferatör reseptör agonisti olan fenofibrat ile karşılaştırılması amaçlandı.

Gereç ve yöntem: Eritrosit lizatlarındaki plazmalojenler ve bazı yağ asitleri GC ile analiz edildi. Plazmadaki çok uzun zincirli yağ asitlerinin yanı sıra fitanik ve pristanik asitleri içeren peroksizomal metabolitler GC-MS ile ölçüldü. Karaciğer kesitlerinde katalaz antikorları ile immünohistolojik analizler gerçekleştirildi.

Bulgular: Test edilen tüm koşullar, kontrollere kıyasla karaciğer kesitlerinde artmış katalaz immünoreaktivitesi gösterdi. Hem kalori kısıtlaması, hem de zaman kısıtlamalı beslenme, ayrıca fenofibrat tedavisi, eritrosit lizatlarında daha düşük C18:0 plazmalojen içeriğini sergiledi. Plazmalogenlerin peroksizomlar tarafından sentezlendiği bilindiğinden, mevcut sonuçlar, eritrosit membranındaki peroksizomal lipit içeriğinin, sirkadiyen ritim bozukluklarından etkilenebileceğini göstermektedir.

Sonuç: Sirkadiyen ritim bozuklukları ve fenofibratın peroksizomal indüksiyonunun eritrosit membran lipidleri üzerindeki ortak etkileri, bunlar arasında bir bağlantı olduğunu göstermektedir.

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Anahtar kelimeler: Kalori kısıtlaması, sirkadiyen ritim, fenofibrat, peroksizom, plazmalojen.

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Introduction

Several studies highlighted the interrelatedness of sleep deprivation, metabolism and circadian disruptions. Seven plasmalogen species were reported to be raised in the rat under acute or chronic sleep restriction conditions. As the plasmalogens are partly synthesized in peroxisomes, the authors pointed out the induction of peroxisome proliferator-activated receptors and disruptions of the circadian clock [1]. However, effects of circadian rhythm disturbances caused by conditions other than sleep restriction have not yet been considered. Restricted feding [2, 3] calorie restriction [4], continuous light exposure [5, 6] metabolic parameters, circadian rhythm activity patterns, and behavior were observed in rats subjected to a 12-h/12-h light/dark cycle (LDand agonists of PPARs, such as fenofibrate [7] have all been reported to cause circadian arythmicity. Currently no reliable biomarker is available to be used in large scale to diagnose circadian disturbances and to monitor the effectiveness of the intervention strategies. To this end, restricted feeding, calorie restriction and continuous light exposure conditions known to interfere with clock rhythm mechanisms in rats were tested for their effect on some peroxisome-related parameters (e.g., plasmalogens, long-chain fatty acids, pristanic acid, and phytanic acid) in blood and liver tissue samples obtained at the termination of 2 weeks of exposure. In order to evaluate the effects of these conditions linked to circadian rhythm disturbances on peroxisomes as well as peroxisomal lipids and to consider their usage as biomarkers. An additional approach was implemented in which rats were fed with fenofibrate (a PPARα agonist) supplemented feeds in order to provoke peroxisomes. Then the results were compared with those of circadian rhythm disturbances tested.

Materials and methods

Experimental design

Seventy five Sprague Drawley male rats weighing 360-380 g were obtained from Inonu University Laboratory Animals Research Center. Inonu University Scientific Ethical Committee on Animal Experimentation approved the study protocol. The animals' care, experimental procedures were carried out by the National Institutes of Health Animal Research Guidelines and ARRIVE guidelines [8]. During the adjustment period for one week, the rats were assigned to five groups, 15 of each, and kept ar 21-22^{o}C under 12:12 hours light-dark cycle and allowed free access to standard rat chow pellet before starting the experiment. Following this period, in the case of time restricted feeding group (TRF), the rats were allowed access to food (rat chow pellet) only between 8:00 a.m. and 11:00 a.m. for two weeks. The rats were allowed daily access to 60% of their normal daily calorie consumption starting at 08:00 am every day in the case of calorie restricted feeding (CRF) experimental condition. Rats in continuous light exposure (CLE) group were kept under continuous light. Additionally, another fifteen of rats were fed with chow pellet contaning 0.1% fenofibrate (Lipanthyl, Reciparm Fontaine or Alembic Pharmaceuticals) (FSD: Fenofibrate Supplemented Diet) for two weeks. Finally, control groups (CTR) were kept under the conditions same as described for the adjustment period. Body weight of rats exposed to various experimental conditions for two weeks were recorded at the commencement and cessation of the experimental procedures.

Collection of the samples

Blood samples obtained from the bifurcation of the femoral artery were collected in tubes containing EDTA. Then the samples were centrifuged at 3.000 rpm for 10 min. at 15^{0}C. The resultant plasma was kept at -80^{0}C until analysed. For plasmalogen analysis, the erythrocyte pellet was washed with an equal volume of saline (0.9% NaCl). The pellets were placed in an Eppendorf tube containing 100µL of 1% butylated hydroxytoluene (BHT) in ethanol dried under the nitrogen stream and kept at -80^{o}C until analysed.

Histopathological analyses

2-3 mm thick liver specimens were taken from the same lobe of the rat liver were fixed in 10% formalin and was embedded in paraffin. Tissue sections were cut at 4μm, mounted on slides, stained with hematoxylin-eosin (H-E) for general liver structure. Hydropic changes in the liver was assessed in 10 randomly selected fields on each section. Alterations in structure were evaluated using a histopathological score as follows: 0, normal; 1, mild; 2, moderate; 3, severe [9].

Immunohistological analyses

Briefly, sections were blocked with 0.3% hydrogen peroxide and incubated with primary catalase antibodies. The sections were then incubated with a biotinylated secondary antibody followed by streptavidin peroxidase and chromogenic substrate AEC. Tissue sections were counterstained with hematoxylin. According to the diffuseness of the staining, the sections were graded as 1=0-25% staining; 2=25-50% staining; 3=staining 51-75%; 4=staining 76-100%. According to the staining intensity, the sections were graded as follows: 0=no staining; 1=weak but detectable staining; 2=distinct; 3=intense staining. Total staining score was obtained as (diffuseness)X(intensity) [10].

Analyses of C16:0 and C18:0 plasmalogens, arachidonic acid (AA) and docosahexaenoic acid (DHA) content in erythrocyte lysates

By adding 3N methanolic HCl to erythrocyte lysates and heating the mixture at 90°C for 4 h, fatty acid glycerol esters are transmethylated resulting in the formation of fatty acid methyl esters whereas the alkyl-1-enyl ether linkage in plasmalogens is cleaved with acidified methanol, leading to the quantitative formation of the fatty aldehyde dimethyl acetals. After cooling the sample, the fatty acid methyl esters and the dimethylacetals are extracted with hexane. Then 1 μl of the resulting hexane solution was injected to GC. 18-methyl-C19:0 was used as the internal standart. The instrumental configuration and analytical conditions were summarized in the following; Shimadzu 2010 GC-FID instrument equipped with a RT-2560 capillary column (100 m x 0.25 mm x 0.20 μm, RESTEK Scientific) and FID Detector. AA and DHA content were expressed as percentages of total fatty acid methyl esters in erythrocyte lysates. The plasmalogen values were not expressed in absolute values, but as a percentage of the level of the corresponding fatty acid. Hence, the C16:0 dimethylacetal is compared with the C16:0 fatty acid methylester, the C18:0 dimethylacetal with the C18:0 fatty acid methyl ester [11].

Very long chain fatty acids, pristanic acid and phytanic acid analyses in plasma

Following alkaline and acid hydrolysis, plasma very long chain free fatty acids, phytanic and pristanic acids extracted with hexane were derivatized with N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide and 1% tert-butyldimethylchlorosilane to tertiarybutyl-dimethylsilyl derivatives. The resultant sample dissolved in hexane were injected into GC-MS (Agilent 6850 GC/Agilent 5977E MS) equipped with a column (Agilent HP5ms, 30 m x 0.25 mm x 0.25 μ). These analyses were conducted in Synlab Türkiye-Laboratory (Ankara/Türkiye).

Quantitative analysis of plasma triacylglycerol

The analysis was carried out spectrophotometrically by Abbott Triacylglyceride Kit via Abbott Architect c16000 automatic analyzer according to the manufacturer's instruction.

Statistical analyses

The sample size of this study was determined by power analysis, G*power 3.1 program. By the priori sample size calculation, the required minimum sample size was calculated as 13 per group for the effect size=0.45 (large) at 95% confidence level (α=0.05) and 80% power (β=0.20). R version 3.5.0 and IBM SPSS Statistics 22.0 software were used for the statistical analyses of triacylglycerol, VLCFA, plasmalogen, immunohistochemical analysis and the rats' body weight measurements. The data were summarized using median, minimum value, maximum value and interquartile range (IQR) statistics. Shapiro-Wilk test was used to determine whether or not the data fit the normal distribution. Kruskal-Wallis H test was used for comparisons between independent groups. Multiple comparison tests were performed with Conover test. Wilcoxon test was used for comparisons between dependent groups. $P<0.05$ was considered to be statistically significant level.

Results

Body weight changes in the rats

Body weight changes among experimental groups were compared. The calorie restricted group lost average 9.6% of their body weight at the end of the experimental period. The decrease was found to be significant. Body weight changes recorded in other groups were found to be insignificant.

Effects of experimental conditions influencing circadian rhythm on some blood lipid parameters

Plasma triacylglycerol levels

In plasma of rats fed on diet supplemented with 0.1% fenofibrate, which is known for its lipid lowering effect, average triacylglycerol concentration was found to be decreased by 66% compared to that of average control values. Whereas calorie restriction in rats resulted in a 45% decrease in average plasma triacylglycerol level compared to the control values. Plasma triacylglycerol levels of TRF and CLE were also lower compared to controls albeit insignificant.

Erythrocyte lysate C16:0 and C18:0 plasmalogen levels and long chain PUFA compositions

The chromatogram of fatty acids and C16:0 and C18:0 plasmalogen is depicted in Figure 1. The percentage C16:0 plasmalogen level in erythrocyte haemolysates of FSD rats was found to be slightly lower compared to that of control values. Whereas plasmalogen levels in other groups were similar to that of control values. In the case of percentage C18 plasmalogen levels in erythrocyte lysates, all but TRF group exhibited significantly lower level in comparison to that of control group. The decrease in all the groups varied from 6% to 10%. FSD and CRF groups showed highest level of decrease in erythrocyte plasmalogen level (Figure 2). AA (C20:4 (n-6)) and DHA (22:6 (n-3) percentages in erythrocyte haemolysate were found to be significantly decreased by 11% and 35% in FSD group respectively compared to that of control group. Both fatty acids were also lower in CRF group however in the case of DHA, the difference was not significant (Figure 2).

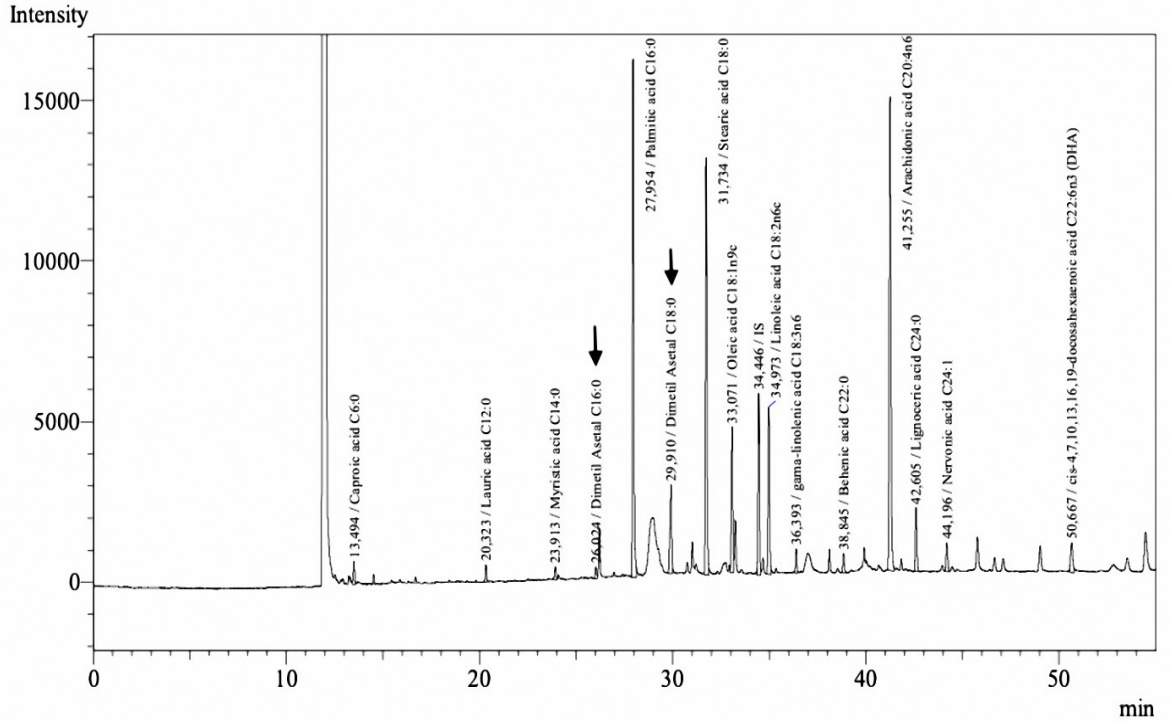

Figure 1. GC chromatogram overlay of fatty acid methyl esters and plasmalogen dimethylacetals in erythrocyte lysates

The plasmalogens can be distinguished next to their corresponding fatty acid methyl esters. Arrows indicate C16:0 or C18:0 plasmalogen dimethylacetals

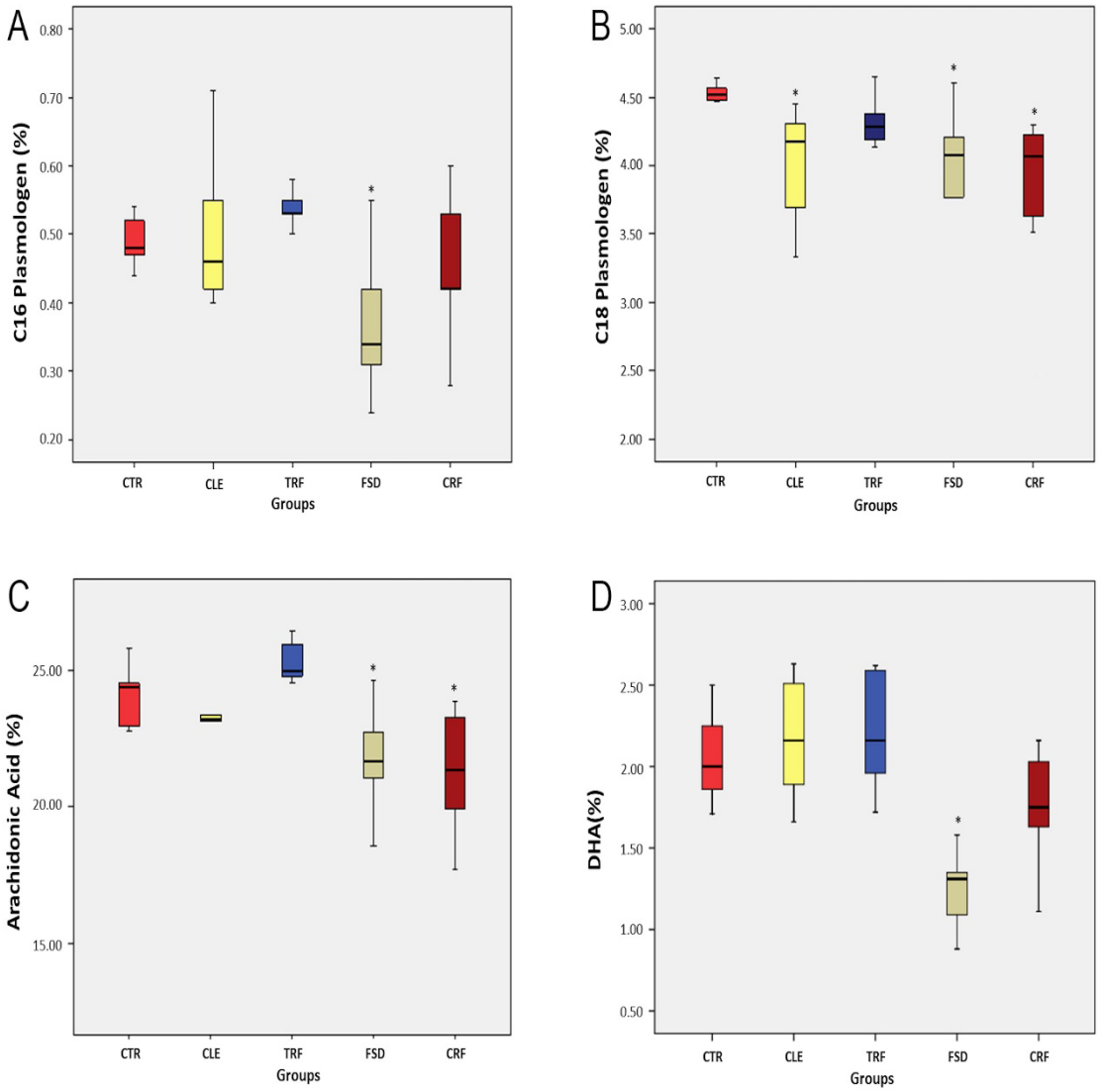

Figure 2. Effects of various experimental conditions associated with circadian disturbances on erythrocyte plasmalogens, arachidonic acid and docosahexaenoic contents

Effects of circadian disturbances on erythrocyte C16:0 plasmalogen content (A), on C18:0 plasmalogen content (B), on arachidonic acid content (C) and on docosahexaenoic acid (DHA) (D). The plasmalogen levels are expressed as a percentage of the level of the corresponding fatty acid. The results of arachidonic acid and docosahexaenoic acid compositions are expressed as percentages of total fatty acid methyl esters. Error bars represent median (n=10), *p<0.05 compared with CTR group

Plasma concentrations of very long chain and branched chain fatty acids

Comparison of C22:0, C24:0 and C26:0 very long chain fatty acids concentrations made among groups or between control group and either of the experimental groups indicated no significant difference (Table 1). However slightly higher C22:0 and C24:0 levels (2-3 nmol/L) was discernable in CRF group compared to the others. In the case of branched chain fatty acids, calorie restriction produced higher level of plasma phytanic acid concentrations whereas fenofibrate treatment yielded lower concentrations compared to the average control value. However, plasma pristanic acid concentrations were found to be similar among all the groups (Table 1).

Table 1. Levels of plasma very long chain fatty acids, pristanic acid and phytanic acid in various circadian disorder conditions of rats

		nmol/mL				
	Group	**Median**	**Minimum**	**Maximum**	**IQR**	***p***
C22:0	**CTR**	6.73	5.34	10.01	1.95	**0.22**
	CLE	7.37	3.12	15.30	6.27	
	TRF	5.92	3.87	10.22	4.28	
	FSD	6.17	3.12	10.60	4.60	
	CRF	8.51	5.72	12.56	4.21	
C24:0	**CTR**	15.28	9.32	21.37	3.50	**0.275**
	CLE	17.3	4.84	29.30	10.9	
	TRF	14.74	9.59	22.46	8.61	
	FSD	13.14	6.76	26.30	9.26	
	CRF	18.13	13.36	27.05	9.17	
C26:0	**CTR**	0.07	0.05	0.10	0.02	**0.49**
	CLE	0.06	0.05	0.10	0.03	
	TRF	0.07	0.05	0.60	0.02	
	FSD	0.06	0.05	0.61	0.02	
	CRF	0.06	0.04	0.09	0.02	
Phytanic Acid	**CTR**	0.44	0.32	0.59	0.12	**0.0001**
	CLE	0.48	0.26	0.96	0.30	
	TRF	0.43	0.29	0.71	0.16	
	FSD*	0.22	0.12	0.36	0.13	
	CRF*	0.58	0.42	0.72	0.16	
Phytanic Acid	**CTR**	6.46	4.76	11.50	3.41	**0.571**
	CLE	6.45	3.05	11.38	3.23	
	TRF	5.59	4.5	9.42	2.88	
	FSD	5.49	3.16	11.10	3.50	
	CRF	5.97	5.15	9.54	1.64	

Data are presented as median, minimum, maximum and IQR. Significance of differences compared with CTR group was indicated as $^{*}p<0.05$ n=12 for each group

Morphology of the liver

Hydropic changes were assessed in 10 randomly selected fields in each liver section and scored (Table 2). The liver sections of CTR group were normal in their histological appearance. Hepatocyte cordons were radially organized around the central vein in an orderly way. Sinusoids associated with hepatocyte cordons were open. In portal areas around liver lobules, arterial, venal and bile canalicular structures were prominently observed. Hepatocytes displayed an eosinophilic cytoplasm with round euchromatic nucleus (Figure 3). Hepatocytes of CRF and TRF groups however, showed marked hydropic changes (p=0.001) (Figure 3.B and C), whereas hydropic changes in that of CLE group was less noticeable. These changes were found to be significant in comparison to histological findings of control liver sections. Fenofibrate supplementation produced no noticable changes in hepatocyte histology. Histopathologic scoring of hydropic changes can be seen in Figure 3.

Table 2. Hydropic changes in hepatocytes and catalase immunoreactivity score results

Groups	Hydropic Changes			Catalase Immunreactivity		
	Median	Minimum	Maximum	Median	Minumum	Maximum
CTR	0.0	0.0	0.0	8.0	4.0	12.0
CLE [a,b]	1.0	0.0	3.0	10.0	4.0	12.0
TRF [a,b]	3.0	0.0	3.0	12.0	4.0	16.0
FSD [b]	0.0	0.0	0.0	3.0	1.0	6.0
CRF [a,b]	2.5	1.0	3.0	12.0	12.0	16.0

Results are expressed as minimum, maximum and median. Total staining score were used as an indication for catalase concentration
The scores are displayed in the table below the pictures, [b]$p \leq 0.001$, compared with CTR group (n:10 for each group)
Scores of hydropic changes can be seen in the [a]$p \leq 0.001$, compared to CTR group

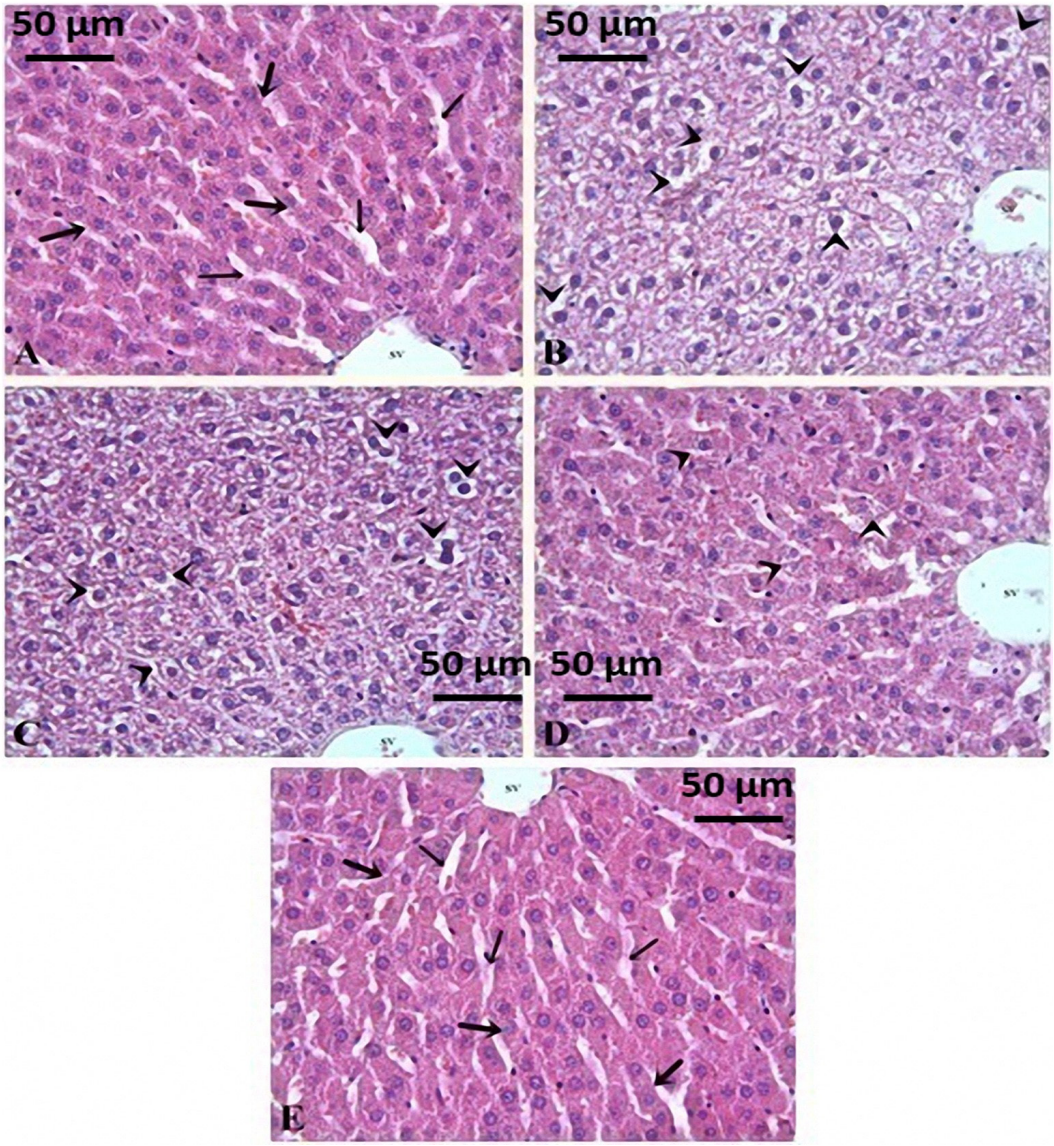

Figure 3. Histopathological picture of H&E-stained sections and scores of hydropic changes in rat livers exposed to various conditions of circadian disruptions

Hydropic changes was assessed in randomly selected 10 fields and scored. (A), A nearly normal liver architecture with CTR group. Various degrees of hydropic changes can be discerned in CRF (B), TRF (C) and CLE (D) groups. Histological features of the liver from FSD group (E) was similar to that of CTR group. SV: Central vein, thick arrows indicate hepatocyte cordons, thin arrows point sinusoids, arrowheads indicate hydropic changes ($p \leq 0.001$, compared with CTR group, n=10 for each group; magnification: x40)

Immunoreactivity of catalase in liver tissue sections of rats

The highest catalase immunoreactivity was observed in liver sections of CRF group whereas the lowest in that of fenofibrate supplemented group. Catalase immunoreactivity of control group had homogeneous distrubition throughout the liver section and was scored as 8 (4.0-12.0) (Table 2). Average catalase scores of CRF, TRF and CLE groups were found to be 12.0, 12.0 and 10.0 respectively being significantly higher as compared to that of control group. As in the case of control group, catalase immunoreactivity in liver sections of these groups exhibited a uniform distribution (Figure 4). On the other hand, heterogenous distrubition of catalase immunoreactivity in liver sections from fenofibrate supplemented group was observed (scored 3.0). The treatment with fibrates induced stronger proliferation of peroxisomes in zone 3 (pericentral hepatocytes) than in zone 1 hepatocytes. Since 10 randomly selected fields on each section was assessed according to the staining intensity, average catalase score of the selected fields became lower than it actually was in fenofibrate treated liver sections.

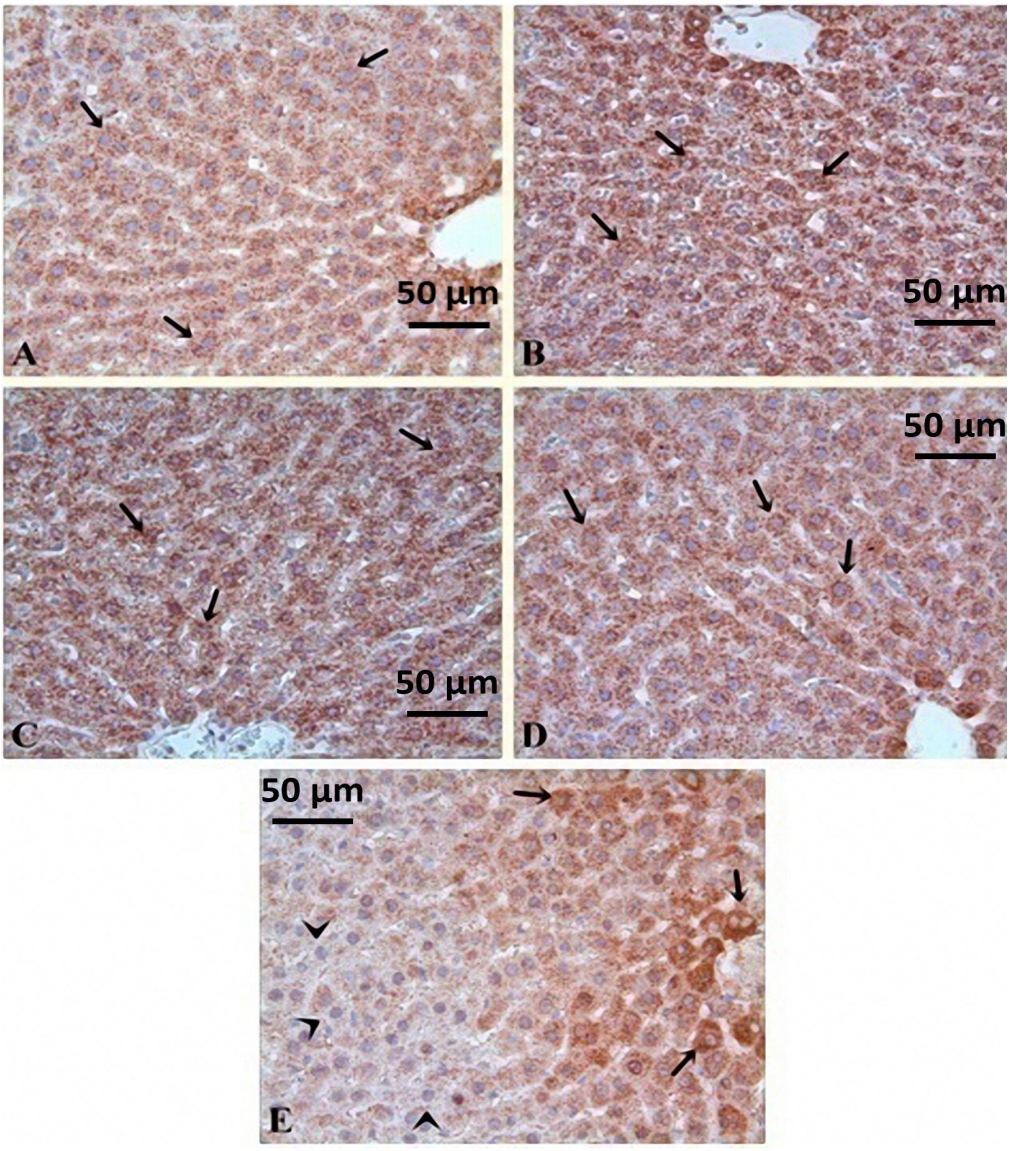

Figure 4. Immunohistochemical reactivity for catalase in livers of rats subjected to various circadian perturbations

Catalase immunoreactivity of CRF (B), TRF (C) and CLE (D) were found to be higher compared to that of the CTR group. These groups and (A) also showed uniform distribution of catalase immunoreactivity. Heterogenous distribution of catalase immunureactivity in liver sections from FSD group are discernable (E) which were more prominent in zone 3. Arrows indicate catalase immunoreactivity. Arrowheads point hepatocytes without catalase immunoreactivity. Total staining score were used as an indication for catalase concentration ($p \leq 0.001$ compared with CTR, n=10 for each group; magnification: x40)

Discussion

Lipids with ether linkage are plasmenyl (plasmalogens) glycerophospholipids that compose approximately 20% of the mammalian total phospholipid pool. Plasmalogens are enriched of AA and DHA in sn-2 position and generally either C16:0 or C18:0 fatty acid is linked to sn-1 position [12, 13]. In the current study, percentage of C18:0 plasmalogen level in erythrocyte lysates were found to be lower in all but TRF group. Lower level of C16:0 plasmalogen level was also detected in FSD group. However, in humans, circadian rhythm disturbances induced by sleep restriction, higher plasma plasmalogen levels were reported [1]. We measured C16:0 and C18:0 plasmalogen levels in erythrocyte lysates representing erythrocyte membranes. Whereas Weljie et al. [1] (2015) measured phospholipids including plasmalogens in human serum. Authors put forward possibility that a source for the elevated phospholipids could be membrane breakdown or release from circulating lipoprotein particles. Accordingly, the discrepancy between our results and that of Weljie [1] can be accounted for by the fact that the release of membrane phospholipids induced by circadian disturbances into serum can cause an increase in serum plasmalogen level and a decrease in membrane plasmalogen level. This possibility requires further research. On the other hand, several plasma plasmalogens were reported to decrease with acute calorie restriction in humans [14]. This discrepancy could be attributed to numerous situations that can affect plasmalogen levels one of which reported to decrease plasmalogen level is lipid oxidation. Plasmalogens are highly susceptible to oxidation [15] and are consumed in this reaction [16]. It is also possible that erythrocyte membrane plasmalogen levels might have been decreased by increased myeloperoxidase which was reported to react with vinyl ether bond of cellular plasmalogens by its ROS [16, 17].

Biochemically, various peroxisome dependent parameters are abnormal in peroxisome deficient mice which include accumulation of VLCFA (because of impaired VLCFA oxidation), lack of plasmalogen, accumulation of phytanic acid, lower DHA in erythrocytes [18]. In peroxisomal biogenesis disorders, VLCFAs accumulate, demonstrating the indispensable nature of peroxisomes in oxidizing these substrates [19]. In our study, none of the conditions of circadian rhythm disturbances tested led to significant variations in plasma levels of very long chain fatty acids (C22:0, C24:0 and C26:0). It is well established that catabolism of phytanic acid, a branched-chain fatty acid, by alpha-oxidation in peroxisomes yields pristanic acid [18]. In the current work, calorie restriction produced higher levels of plasma phytanic acid. This might suggest a slowing in α-oxidation of peroxisomes due to calorie restriction. On the other hand, fenofibrate supplementation resulted in lower plasma levels of phytanic acid and triacylglycerol levels indicating occurence of peroxisomal induction by fenofibrates as expected. Overall our data points out that calorie restriction might lower peroxisomal oxidation whereas fenofibrates enhance it. Calorie restriction and fenofibrates appear to have diverse effects on α-oxidation in peroxisomes.

Hepatocytes of CRF and TRF groups showed marked hydropic changes, whereas hydropic changes in that of CLE group was less pronounced. It is possible that excess lipolysis resulting from calorie restriction might have caused lipotoxicity [20-22] and subsequent hydropic changes in liver sections. On the contrary, fenofibrate supplementation produced no noticable hydropic changes in liver tissue sections. PPAR alpha agonists are known to suppress inflammatory events via the NF-κB pathway and inhibit ROS production [23, 24], Therefore, it is possible that damaging effect of hydrogen peroxide by-product of peroxisomal oxidation activity might have been served as an offset through antioxidant and antiinflammatory action of fibrates.

The most highly expressed and best characterized peroxisomal antioxidant enzyme is catalase [25]. Catalase activity is largely or completely located in peroxisomes [26]. Calorie restriction or body weight loss have been reported to be associated with enhanced catalase activity [23, 27, 28]. Increased peroxisomal β-oxidation and as a result, increased production of hydrogen peroxide at peroxisomal acyl CoA oxidase stage might have been the cause of high uniform catalase activity seen in the experimental groups other than FSD in our work. It has been reported that treatment with fibrates induced stronger proliferation

of peroxisomes in zone 3 rather than in zone 1 hepatocytes [29] which is in line with our findings in FSD group indicating heterogenously increased catalase immunoreactivity in liver sections. It is known that CRF increased the expression of the genes which involve in fatty acid metabolism and PPAR signalling pathway [30]. It is possible that excess lipolysis resulting from calorie restriction might have caused lipotoxicity [20-22] and subsequent hydropic changes in liver sections. On the contrary it is reported that TRF may attenuate the lipid homoeostasis in liver by regulating the clock components [31]. Moreover, fenofibrate supplementation produced no noticable hydropic changes in liver tissue sections.

The present results might imply that altered feeding regiments and fenofibrates might influence different subpopulation of peroxisomes. Both calorie restriction and fenofibrate treatments were reported to have induced changes in fatty acid compositions in tissues. Some workers reported a decrease in relative amount of arachidonic acid in membrane phospholipids [32, 33]. On the other hand, treatment with fibrates was reported to induce shifts in fatty acid composition [34] including EPA and DHA contents in tissues [35]. Our findings also indicate lower arachidonic acid and DHA contents in erythrocytes of FSD and CRD group. Arachidonic acid is the major essential fatty acid component of membrane phospholipids. Enzymatic breakdown of arachidonic acid yields a variety of bioactive lipid molecules that have diverse physiological roles in many cellular processes [36] which might be mediators in metabolic disorders with concurrent circadian disruptions.

Consequently, some common effects of the various conditions co-occuring with circadian rhythm disturbances and fenofibrate inducement on the C-18 plasmalogen contents in erythrocyte membranes and on catalase activity in liver sections might point out a link between peroxisomal function and circadian rhythm disturbances. Evaluation of peroxisomal lipids in erythrocyte membranes could be a new tool for the diagnosis of circadian rhythm disturbances.

In the present study, the effect of acute circadian rhythm disturbances on membrane lipid composition was examined and the short duration of the application period was a limitation in the evaluation of the results. The short-term rhythm disturbance model did not produce significant changes on the erythrocyte membrane, and the chronic rhythm disturbance model may give more meaningful results in determining the effect of long-term rhythm disturbances. In addition, since circadian rhythm shows individual differences, we believe that comparisons within individuals will give more accurate results rather than evaluating on a group basis.

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Author contributions

T.G. wrote manuscript and performed data acquisition. H.G.O. performed the experiments and acquisition of the data. S.E., Y.U., A.Y. and N.V. performed part of the experiments and methodology. S.Y. carried out statistical analyses. All authors read and approved the final manuscript.