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ORIGINAL ARTICLE

Daily rhythms of lipid metabolic gene expression in zebra fish liver: Response to light/dark and feeding cycles

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Despite numerous studies about fish nutrition and lipid metabolism, very little is known about the daily rhythm expression of lipogenesis and lipolysis genes. This research aimed to investigate the existence of daily rhythm expressions of the genes involved in lipid metabolism and their synchronization to different light/dark (LD) and feeding cycles in zebra fish liver. For this purpose, three groups of zebra fish were submitted to a 12:12 h LD cycle. A single daily meal was provided to each group at various times: in the middle of the light phase (ML); in the middle of the dark phase (MD); at random times. After 20 days of acclimation to these experimental conditions, liver samples were collected every 4 h in one 24-h cycle. The results revealed that most genes displayed a significant daily rhythm with an acrophase of expression in the dark phase. The acrophase of lipolytic genes (lipoprotein lipase – *lpl*, peroxisome proliferator-activated receptor – *ppar*α and hydroxyacil CoA dehydrogenase – *hadh*) was displayed between ZT 02:17 h and ZT 18:31 h. That of lipogenic genes (leptin-a – *lepa*, peroxisome proliferator-activated receptor – *ppar*γ, liver X receptor – *lxr*, insulin-like growth factor – *igf1*, sterol regulatory element-binding protein – *srebp* and fatty acid synthase – *fas*) was displayed between ZT 15:25 h and 20:06 h (dark phase). Feeding time barely influenced daily expression rhythms, except for *lxr* in the MD group, whose acrophase shifted by about 14 h compared with the ML group (ZT 04:31 h versus ZT 18:29 h, respectively). These results evidence a strong synchronization to the LD cycle, but not to feeding time, and most genes showed a nocturnal acrophase. These findings highlight the importance of considering light and feeding time to optimize lipid metabolism and feeding protocols in fish farming.

Keywords: *Danio rerio*, lipogenesis, lipolysis

INTRODUCTION

The Earth's rotation generates a predictable environment of natural cyclic changes of light and temperature. This natural pressure has fostered the evolution of biological clocks that keep track of time, and provide organisms with an anticipatory temporal framework for optimal physiological and behavioral activities. The mechanism of these clocks requires daily adjustments, achieved through the input provided by environmental factors or synchronizers, the most important of which are light/dark (LD) (Panda et al., 2002) , temperature (Rensing & Ruoff, 2002) and feeding (Mistlberger, 2009) cycles.

A biological process's rhythmicity is explained by the existence of a self-sustained pacemaker. In vertebrates, the most important is the central pacemaker, also called the light-entrainable oscillator (LEO), which is synchronized by LD cycles. In mammals, reptiles and birds, a master circadian LEO has been found in the suprachiasmatic nucleus of the hypothalamus (SCN) (Bertolucci et al., 2008; Shibata & Tominaga, 1991; Welsh et al., 2010). The existence of a food-entrainable oscillator (FEO) in vertebrates has also been hypothesized, but its anatomical location remains unknown, although some hypothalamic and brainstem nuclei locations have been suggested (Blum et al., 2012; Challet et al., 2009; Davidson 2009). In fish, evidence indicates the presence of these two different oscillators (light-entrainable oscillator – LEO and FEO), although their exact location and relationship are still not completely understood (López-Olmeda et al., 2010).

Circadian regulation controls most physiological activities during the course of a day (Panda et al., 2002). Recent comparative analyses of the

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transcriptome, metabolome and proteome have revealed clock-dependant control in a number of metabolic pathways in the mouse liver (Eckel-Mahan et al., 2012; Mauvoisin et al., 2014; Panda et al., 2002; Robles et al., 2014; Storch et al., 2002). Such clock regulation covers multiple activities like lipogenesis, xenobiotic detoxification, cholesterol synthesis, ribosome biogenesis, mitochondrial respiration, sleep-wake rhythms, hormone secretion or cognitive tasks (Dibner et al., 2010; Gerstner et al., 2009; Jouffe et al., 2013; Mauvoisin et al., 2014; Menet & Rosbash, 2011; Peek et al., 2013; Sahar & Sassone-Corsi, 2012). In mammals, the lipid metabolism displays rhythms controlled by a circadian clock (Alila-Johansson et al., 2004; Bertolucci et al., 2008; Bitman et al., 1990; Escobar et al., 1998; Piccione et al., 2003). Growing evidence suggests that the central pacemaker controls daily rhythms of peripheral tissues through output cues, such as systemic signals (Bass & Takahashi, 2010; Masri & Sassone-Corsi, 2010), to hence orchestrate the clock system as a whole. Interestingly, the hepatocyte circadian clock is highly susceptible to factors other than the LD cycle; e.g. variations in nutrient composition like a high-fat diet (Eckel-Mahan et al., 2013; Janich et al., 2014; Pendergast et al., 2013) and food intake timing, and to the extent that antagonistic feeding times (mid-light food intake versus mid-dark food intake) reset the liver peripheral clock almost completely, and induce alterations in rhythmic gene expressions and circadian phase changes (Arble et al., 2009; Damiola et al., 2000; Hughes et al., 2009; Paredes et al., 2014; Stokkan et al., 2001; Vollmers et al., 2009; Yoshida et al., 2012). These alterations appear in clock gene expressions in the liver (peripheral oscillator), which is synchronized by feeding time, but not in the brain (central pacemaker), which is synchronized by the LD cycle (Vera et al., 2013). Therefore, it is necessary to study how feeding time affects gene expression in the liver in more depth in order to understand the cooperative forces that operate between the core clock system and its integration into systemic signal controlling tissue-specific oscillations caused, for instance, by different food intake timing. Such studies are necessary in order to prevent disruptions in the circadian clock that might lead to pathologies such as obesity, neurological diseases and cancer (Janich et al., 2014).

Lipid metabolism in fish can be studied at the molecular level by referring to the expression of lipogenesis and lipolysis genes. Several of these genes play an essential role in lipogenesis: liver X receptor (*lxr*), which regulates the transcription of the genes involved in fatty acid synthesis (Repa et al., 2000; Schultz et al., 2000), cholesterol homeostasis (Zhang & Mangelsdorf, 2002; Steffensen & Gustafsson, 2004; Tontonoz & Mangelsdorf, 2003), and carbohydrate metabolism (Laffitte et al., 2003; Mitro et al., 2007); sterol regulatory element-binding protein (*srebp*), which regulates the genes involved in sterol biosynthesis and fatty acid synthesis (Horton et al., 2002); peroxisome proliferator-activated receptor-γ (*pparγ*), a critical gene in adipogenesis (Grimaldi et al., 2010) and triglyceride metabolism (Rivera-Zavala et al., 2011); fatty acid synthase (*fas*); insulin-like growth factor 1 (*igf1*), involved in growth processes and signals in lipid metabolism, such as *pparγ* and *srebp* (Piccinetti et al., 2013); leptin-a (*lepa*), a satiety hormone implicated in energy balance and glucose homeostasis. Some genes have been described to play an essential role in lipolysis in fish, such as lipoprotein lipase (*lpl*), which facilitates the uptake of fatty acid and its oxidation or storage as triglycerides (Saera-Vila et al., 2007); peroxisome proliferator-activated receptor α (*pparα*), which fosters fatty acid oxidation (Szántoóvá et al., 2011); hydroxyacyl CoA dehydrogenase (*hadh*), which mediates in fatty acid metabolism by catalyzing the third step in beta-oxidation (Schulz et al., 2011).

The aim of this research was to elucidate the existence of a daily rhythm of the expression of the genes involved in lipid metabolism and their synchronization to LD (12 L:12D) and feeding cycles in zebra fish liver. Three different feeding regimes were used: a single daily meal delivered at mid-light (ML), mid-darkness (MD) or random feeding (RD) time points. The synchronization of daily behavioral rhythms (locomotor activity) to light and feeding times was also investigated.

MATERIAL AND METHODS

Animals and housing

Wild-type zebra fishes ($N = 90$; 0.62 ± 0.20 g body weight) were housed at the Fish Chronobiology Laboratory of the University of Murcia in a 9-l glass aquaria. A photoperiod was set at a 12 h:12 h LD cycle, with lights on (Zeitgeber time 0, ZT0) at 9.00 a.m. Light was provided by LED lamps with a light intensity on the water surface of 0.84 W m^{-2} (200 lx). Water temperature was controlled at 26 °C throughout the acclimation and experimental periods. Fishes were fed with commercial feed (Tropical Fish Flakes, Casone, Parma, Italy).

Experimental design

Fishes were reared and manipulated following Spanish legislation on Animal Welfare and Laboratory Practices. Experimental protocols were performed following the Guidelines of the European Union (2010/63/UE) and Spanish legislation (Royal Decree 1201/2005 and Law 32/2007) for the use of laboratory animals, and were approved by the National Committee and the Ethics and Animal Welfare Committee of the University of Murcia (Spain). The experiments fulfilled the ethical standards required by the journal (Portaluppi et al., 2010).

Zebra fish were divided into three groups and were fed once a day at different times: in the middle of the light phase (ML), in the middle of the dark phase (MD) or at random times (RD). In each experimental group, fish were divided into six tanks per feeding regime (five fish per tank, total $N = 30$) so that each tank was

TABLE 1. Gene names and functions.

Gene	Name	Gene function
Lepa	Leptin-a	Satiety hormone. Energy balance and glucose homeostasis
srebp	Sterol regulatory element-binding protein	Regulates genes involved in sterol biosynthesis and fatty acid synthesis
lxr	Liver X receptor	Prolipogenic functions
Igf1	Insulin-like growth factor	Growth process and signals involved in lipid metabolism (*ppar*γ and *srebp)*
pparγ	Peroxisome proliferator-activated receptor-γ	Nuclear receptor critical in adipogenesis, insulin sensitivity, and inflammatory response
fas	Fatty acid synthase	Fatty acid synthesis
lpl	Lipoprotein lipase	Facilitates the uptake of fatty acids and their oxidation or storage as triglycerides
pparα	Peroxisome proliferator-activated receptor-α	Foster fatty acid oxidation
hadh	Hydroxyacil CoA deshydrogenase	Involved in fatty acid metabolic processes Catalyzes the third step in the beta-oxidation

sampled at a different sampling time. Food was provided by an automatic feeder (Eheim GmbH & Co. KG, model 3581, Deizisau, Germany). The feeders of the ML and MD groups were set to deliver food at the same time every day (ZT6 and ZT18 for ML and MD, respectively), whereas food was delivered randomly once a day to the RD group. The feeding interval for the RD group was set between 12 and 36 h. So on average, the RD group received the same amount of food every 24 h as the ML and MD groups. Feeders were adjusted to provide food at 1.5% of fish body weight.

Locomotor activity was measured by an infrared photocell (E3Z-D67, Omron, Kyoto, Japan), which was placed under the feeder in each aquarium, 3 cm below the water surface. Each photocell was connected to a computer, which counted and stored the number of light beam interruptions at 10 min intervals with specialized software (DIO96USB, University of Murcia, Murcia, Spain).

After 20 days of being fed according to the different feeding regimes (ML, MD and RD), zebra fish fasted for 24 h and were then sampled at ZT1, 5, 9, 13, 17 and 21 h. The fish ($n = 5$) from one tank per group were sampled at each time point. Fish were killed by submersion in ice water (5 parts ice/1 part water, 0–4 °C). Liver samples were collected, frozen immediately in dry ice and stored at −80 °C until processed. Sampling in the dark phase was performed under a dim red light ($\lambda > 600$ nm).

Gene expression assays

Liver was homogenized using Trizol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA) with a tissue homogenizer (POLYTRON, PT1200, Kinematica, Lucerne, Switzerland). Total RNA purity and concentration were determined by spectrometry (Nanodrop ND-1000, Thermo Fisher Scientific Inc., Wilmington, DE). Then, 1 µg of RNA was treated with amplification grade DNase I (1 unit/µg RNA, Invitrogen) to prevent genomic DNA contamination, and was retrotranscribed with the QuantiTect Reverse Transcription kit (Qiagen, Venlo, The Netherlands).

For this study, we selected a number of genes which play key roles in lipid metabolism processes as regards lipogenesis–lipolysis (Table 1). Real-time quantitative PCR was performed using the SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) in an ABI Prism 7500 apparatus (Applied Biosystems) according to the following protocol: 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. Each PCR well contained a final 20 µl volume: 5 µl of cDNA, 10 µl of the qPCR Master Mix and 5 µl of each forward and reverse-specific primer. Samples were run in duplicate. The primers of each gene were designed with Primer3Plus (Untergasser et al., 2012) and were tested to verify their efficiency by means of a standard curve (Table 2). Reaction specificity was validated by analyzing the melting curve. The relative expression of all the genes was calculated by the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001) using *Danio rerio* elongation factor 1 alpha (*ef1*α) as a housekeeping gene.

Data analysis

Fish locomotor activity was analyzed and rendered in actograms by the El Temps chronobiology software (v.1.275, Dr. Díez-Noguera, University of Barcelona, Spain). The cosinor analysis (CSR software, 3.0.2) was performed to determine whether the daily expression of the studied genes fitted cosine function $Y = M + A * [\mathrm{Cos}(\Omega\tau + \Phi)]$ to reveal the existence of any statistically significant daily rhythm, where M is mesor, A is amplitude, Ω is angular frequency (360°/24 h for circadian rhythms), τ is the time period (24 h) and Φ is the acrophase. The cosinor analysis also provided a statistical value for the null hypothesis of zero amplitude; for a statistical significance of $p < 0.05$, the null hypothesis was rejected, and amplitude was considered different from zero.

The statistical differences of the lipid gene expression between the various sampling times were subjected to a one-way ANOVA, followed by Tukey's *post hoc* test. Each gene from each feeding regime was independently analyzed. ANOVA tests were performed with the SPSS

TABLE 2. Zebrafish primer sequence for real-time PCR.

Gene	Gene name	F/R	Primer sequence (5′–3′)
fas	ENSDARG00000087657	F	AAGGTCTTTTGCGTCTGCTG
		R	TGCTGTTTTCAAGCGCAGTG
Lpl	ENSDARG00000087697	F	AAACAGCACCGTGTCTTTCC
		R	TTGCGAATGTGGAAGGTGTC
srebp	ENSDARG00000063438	F	TCCTCCATCAACGACAAGATCC
		R	ACACACACGCTGACTTGTTC
lxr	ENSDARG00000043170	F	AGCTGCTTCCTTTGAACGTG
		R	TCGCCAAAACCTGCTTGATG
igf1	ENSDARG00000094132	F	TCCGTCTCCTGTTCGCTAAATC
		R	TTTGGGTCTCCAGCAAAAGC
pparγ	ENSDARG00000031848	F	AGACAAAGCTTCGGGGTTTC
		R	AATCGCGTTGTGTGACATGC
pparα	ENSDARG00000031777	F	CTTCGTCATTCACGACATGG
		R	AAGCGTACTGGCAGAAAAGG
lepa	ENSDARG00000091085	F	AACTGCAGGCAAAGACCATC
		R	GCGGGAATCTCTGGATAATG
hadh	ENSDARG00000060594	F	TGACATTGGTGCCGTTTTCG
		R	TGACAAGGGGTAAACTGGTTCC
ef-1	ENSDARG00000020850	F	CCTTCGTCCCAATTTCAGG
		R	CCTTGAACCAGCCCATGT

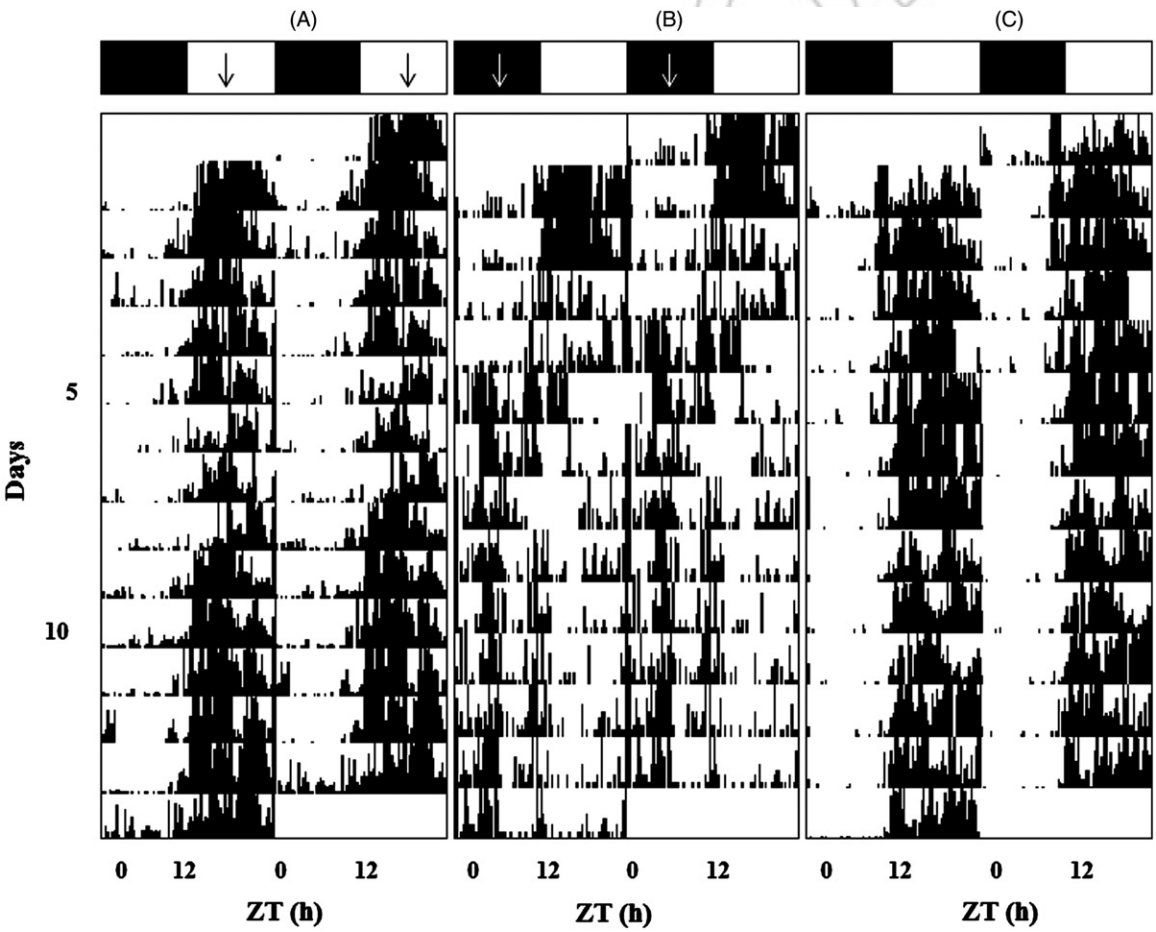

FIGURE 1. Representative actograms of activity from the zebra fish in a 12:12 LD cycle, fed once a day at ML (A), MD (B) or RD times (C). For convenient viewing, the data were double-plotted (48 h), the *y*-axis progresses in single days with each day plotted twice (day 1 on the right side is repeated on day 2, on the left side). Activity was binned every 10 min, and the height of each point represents the number of interruptions of the infrared light beam. The bars above each actogram represent the light regime; open and black bars represent light and dark, respectively, of the LD cycle. The arrows at the top of the actograms indicate the feeding time for the groups with fixed feeding times (ML and MD).

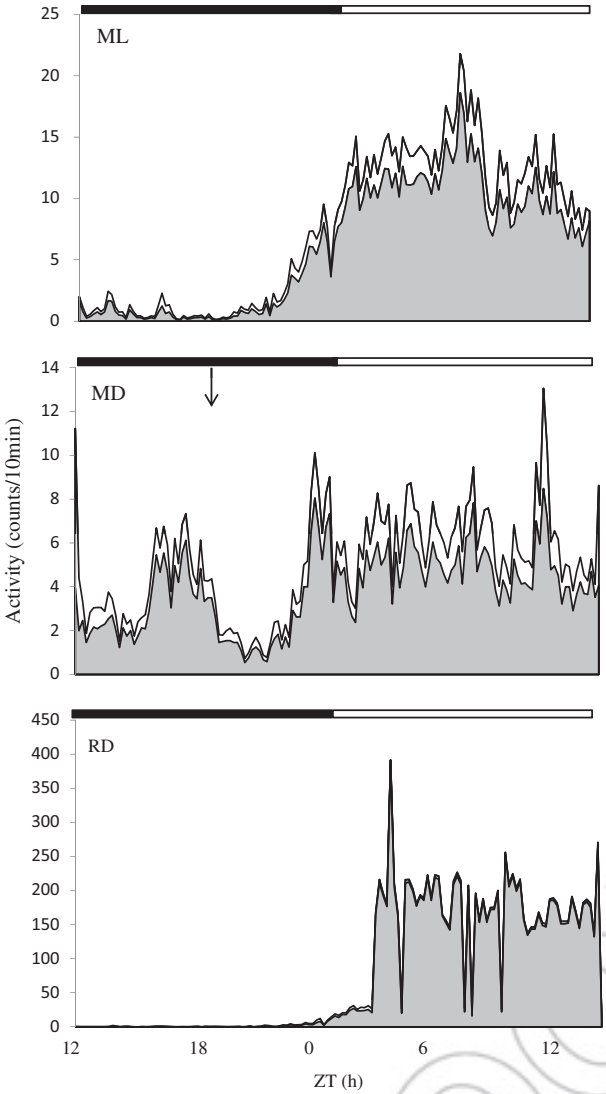

FIGURE 2. Mean waveforms of locomotor activity from the zebra fish in a 12:12 LD cycle fed once a day at ML (A), MD (B) or RD times (C). Each point was calculated as the mean ± SD of 10-min binned data across all the experimental days shown in each actogram in Figure 1. The gray area indicates the mean light beam interruptions, while the continuous line and white area indicate the SD. The bars above the mean waves represent the light (open bars) and dark (black bars) phases of the LD cycle. The black arrows in the ML and MD groups indicate mealtimes.

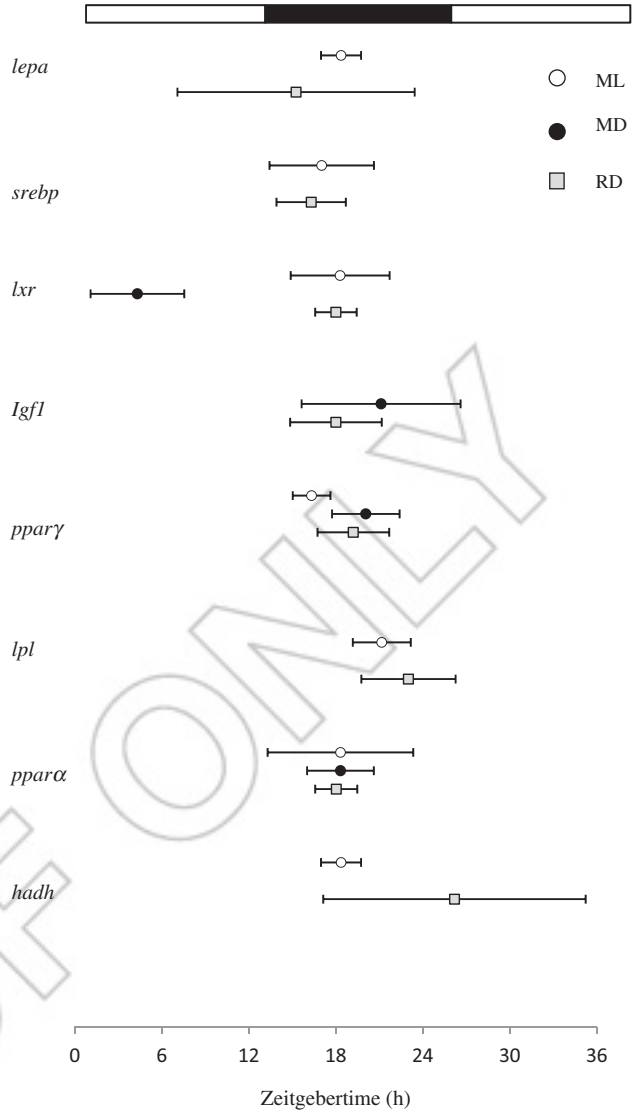

FIGURE 3. Map of the acrophases of the genes analyzed in this research. The acrophase is indicated by different symbols according to the experimental group: white circles for the ML group, black circles for the MD group and gray squares for the RD group. The fiducial limits (set at 95%) are indicated by the lateral bars. The name of each analyzed factor is indicated on the left of the graph. The white and black bars above the graph represent the light period and the dark period, respectively.

v.19 software (IBM, Armonk, NY). Level of significance was set at $p < 0.05$ for all the statistical analyses.

RESULTS

Locomotor activity rhythm

The daily activity rhythms of zebra fish varied depending on the feeding regime. The fish fed at ML or RD displayed a clear diurnal activity rhythm with 88.57 and 90.08%, respectively, of their activity being displayed in the light phase of the LD cycle. Those fed at MD exhibited lower diurnal activity, with 62.54% of their activity displayed in the light phase (Figure 1). The zebra fish exposed to feeding cycles (i.e. ML or MD) displayed food anticipatory activity (FAA), defined as an increase in locomotor activity over several hours (2–3 h) before mealtime, followed by a decrease after feeding (Figure 2). The RD group, however, failed to show FAA and fish synchronized only to the LD cycle and displayed a diurnal pattern.

Daily rhythms of the expression of lipid metabolism genes

The expression of most of the studied genes displayed a statistically significant daily rhythm (Cosinor $p < 0.05$), and nearly all their acrophases were located in the dark

TABLE 3. Cosinor analysis board for hepatic genes of zebrafish fed at ML, MD and at RD during an LD cycle.

Gene	Feeding time	Significance (P)	Mesor (fold change)	Amplitude (fold change)	Acrophase (ZT hours)
lepa	ML	*	18.6 ± 5.3	23.0 ± 9.0	18:35 ± 1:38
	MD	NS	–	–	–
	RD	*	6.0 ± 3.6	7.3 ± 6.4	15:25 ± 8:37
srebp	ML	*	5.9 ± 3.0	6.2 ± 5.3	17:19 ± 3:57
	MD	NS	–	–	–
	RD	**	12.2 ± 5.5	15.5 ± 10.0	16:29 ± 2:39
lxr	ML	*	1.3 ± 0.4	0.8 ± 0.7	18:29 ± 3:41
	MD	**	1.0 ± 0.3	0.6 ± 0.4	04:31 ± 3:23
	RD	**	4.4 ± 1.1	4.2 ± 1.9	18:00 ± 1:43
igf1	ML	NS	–	–	–
	MD	**	4.0 ± 1.7	3.1 ± 3.0	21:11 ± 5:48
	RD	**	11.9 ± 3.9	9.8 ± 6.8	18:00 ± 3:16
pparγ	ML	**	39.8 ± 11.6	52.8 ± 21.6	16:32 ± 1:30
	MD	**	5.3 ± 1.5	4.3 ± 2.6	20:06 ± 2:33
	RD	**	8.8 ± 2.1	6.0 ± 3.7	19:20 ± 2:47
fas	ML	NS	–	–	–
	MD	NS	–	–	–
	RD	NS	–	–	–
lpl	ML	**	14.4 ± 1.4	4.5 ± 2.6	21:16 ± 2:00
	MD	NS	–	–	–
	RD	*	4.8 ± 2.4	5.6 ± 4.4	23:30 ± 3:25
pparα	ML	*	3.28 ± 1.2	2.2 ± 2.2	18:31 ± 5:02
	MD	**	3.8 ± 1.2	3.0 ± 2.1	18:31 ± 2:30
	RD	**	132.7 ± 47.6	178.8 ± 82.2	18:02 ± 1:45
hadh	ML	**	18.6 ± 5.3	23.0 ± 9.0	18:35 ± 1:38
	MD	NS	–	–	–
	RD	**	4.0 ± 1.5	4.1 ± 2.8	02:17 ± 9:05

NS, Non-significant.
*$p < 0.05$, **$p < 0.01$.
Errors are the mean ± fiducial limits set at 95%.

phase (Figure 3, Table 3), regardless of the feeding regime.

The lipogenic genes analyzed (*lepa, srebp, lxr, igf1, pparγ* and *fas*) in the zebra fish liver showed significant rhythmicity, whose expression peak (Figures 3 and 4) and acrophase (Table 3) were displayed between ZT 15:25 h and 20:06 h (dark phase). However, the acrophase of the rhythmic expression of *lxr* in the MD group was observed in the light phase (ZT 4:31 h). Interestingly, *lxr* expression displayed a nocturnal rhythm in the ML and RD groups, with the acrophase located at mid-dark (ZT 18:29 h and 18:00 h, respectively). The ML group exhibited significant rhythms of *lepa, srebp, lxr* and *pparγ*, the RD group presented significant rhythms of *lepa, srebp, lxr, igf* and *pparγ*, as did MD group for *lxr, igf* and *pparγ*. The expression of *fas* displayed no significant rhythmicity in any of the study groups (Cosinor, $p > 0.05$) (Figures 3 and 4, Table 3). Besides the existence of significant daily rhythmicity, most genes also showed statistically significant differences between the time points (one-way ANOVA, $p < 0.05$) (Figure 4).

The genes associated with lipolytic metabolism (*lpl, pparα* and *hadh*) (Figure 5) displayed significant daily rhythms of expression with an acrophase in the dark phase between ZT 18:31 h and ZT 02:17 h (Figures 3 and 5, Table 3). The expression of *pparα* displayed a similar profile in all three groups: a significant daily rhythm with an acrophase around MD (ZT 18:02–18:31 h). As regards *lpl* and *hadh,* the other two lipolytic genes, significant rhythmicity was observed only in the animals fed at ML and randomly (RD). Regardless of significant rhythmicity existing, significant differences were found for the expression levels of the three studied lipolytic genes among the time points in all three groups (one-way ANOVA, $p < 0.05$) (Figure 5).

DISCUSSION

The control mechanisms of liver lipid metabolism in vertebrates have been the objective of much research for many years. Nevertheless, their rhythmic nature and synchronization to light and feeding cycles are far from being fully understood. Overall, the maximum expression peaks of the analyzed lipogenic metabolic genes (*lepa, srebp, lxr, igf* and *pparγ*) were observed in the dark phase, while the genes linked to lipolytic metabolism (*lpl, pparα* and *hadh*) showed higher expression levels around the second half of the dark phase of the LD cycle. Interestingly, light appeared as the dominant synchronizer in the studied genes rather than other zeitgebers, such as feeding time, except for *lxr*, whose rhythms were shifted by the feeding schedule.

The activity rhythm in the ML and MD groups synchronized to the feeding regime and showed FAA, unlike RD which did not. This result is consistent with

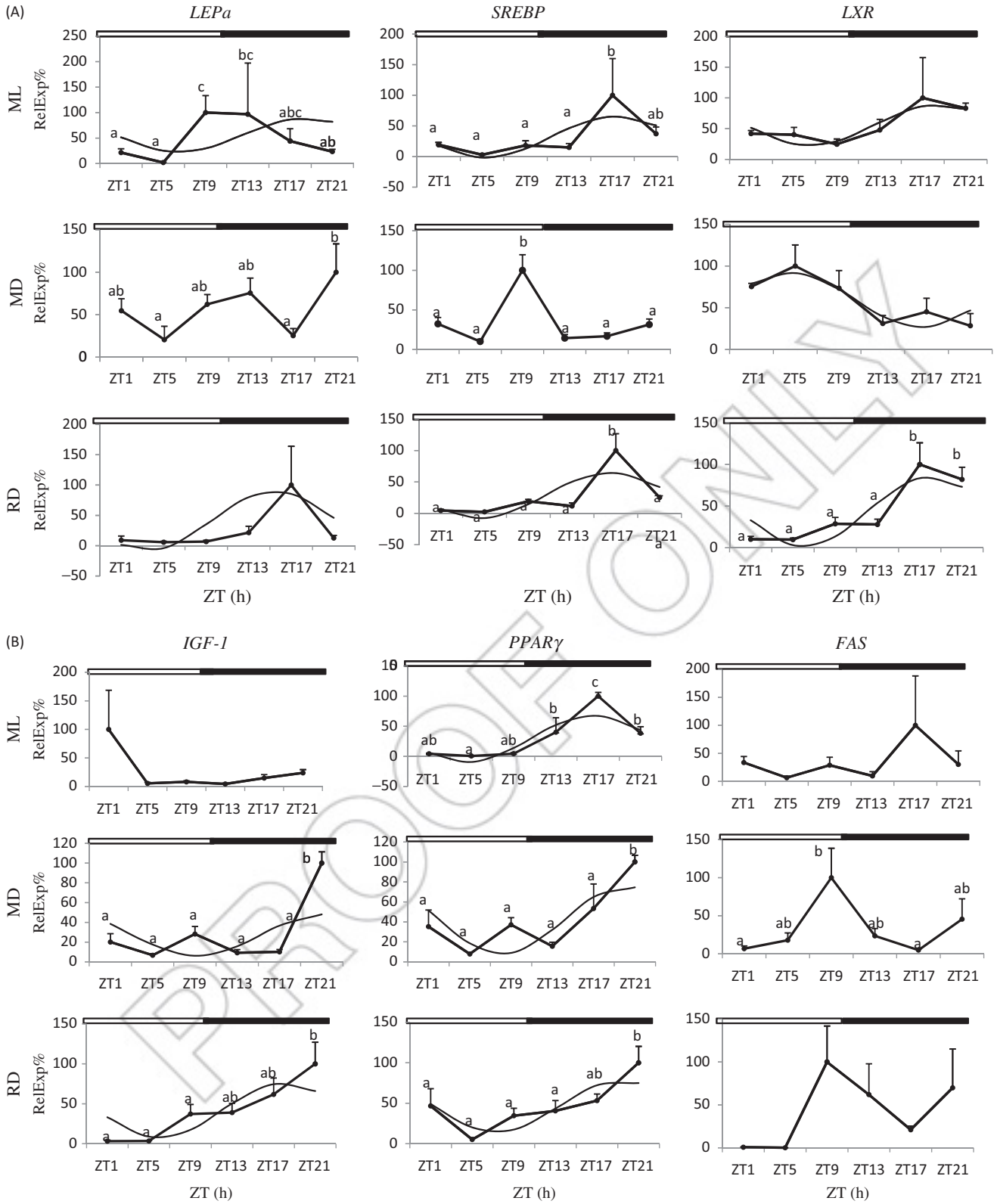

FIGURE 4. The relative expression values (in %) of the lipogenic hepatic genes (*lepa, srebp, lxr, igf, pparγ* and *fas*) in the zebra fish fed at ML, MD and RD. The white and black bars above each graph represent the light period and the dark period, respectively. The sinusoidal line represents the adjustment to a sinusoidal rhythm calculated by the Cosinor analysis whenever this analysis was statistically significant ($p<0.05$). Different letters indicate the various subgroups identified by Tukey's *post hoc* test. Different sampling points, within each group and gene, present statistically significant differences when they do not share any letter (one-way ANOVA, $p<0.05$).

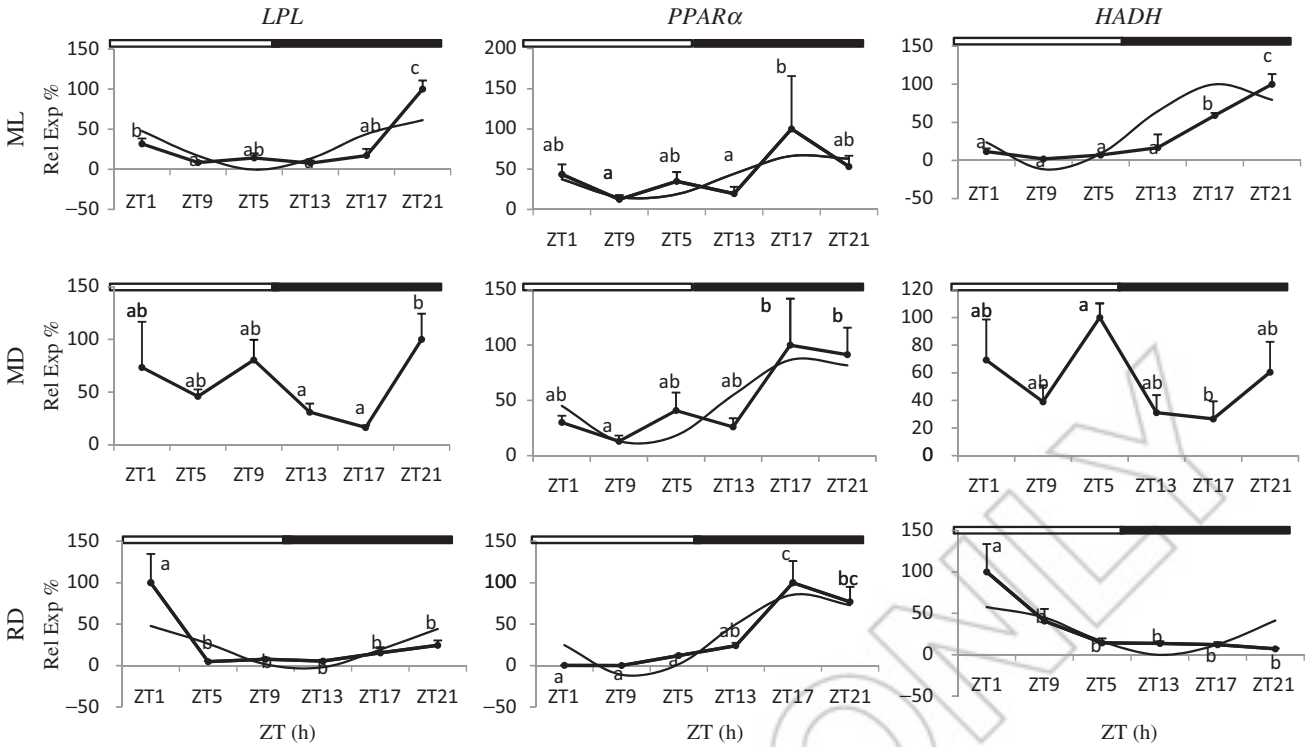

FIGURE 5. The relative expression values (in %) of the lipolytic hepatic genes (*lpl*, *pparα* and *hadh*) in the zebra fish fed at ML, MD and RD. The white and black bars above each graph represent the light period and the dark period, respectively. The sinusoidal line represents the adjustment to a sinusoidal rhythm calculated by the Cosinor analysis whenever this analysis was statistically significant ($p < 0.05$). Different letters indicate the various subgroups identified by Tukey's *post hoc* test. Different sampling points, within each group and gene, present statistically significant differences when they do not share any letter (one-way ANOVA, $p < 0.05$).

the behavioral shift toward nocturnal activity reported previously in other fish species fed at night (Azzaydi et al., 2007; Feliciano et al., 2011; López-Olmeda et al., 2010; Montoya et al., 2010a, b; Sánchez-Vázquez et al., 2001; Vera et al., 2007). The results of the above works revealed the existence of a ''natural'' diurnal activity rhythm in zebra fish with an activity shift toward nocturnalism when fed at night. Recent data have also described zebra fish activity as a mechanism with high plasticity whose pattern, in specific circumstances (mid-dark feeding), can shift toward night activity (del Pozo et al., 2011; López-Olmeda et al., 2010). The display of FAA reveals the presence of an ancestral evolutionary response that provides the ability to anticipate a forthcoming meal by modulating behavioral and physiological outputs to improve nutrient utilization (Comperatore & Stephan, 1987; López-Olmeda et al., 2010; Strubbe & Van Dijk, 2002).

Considerable evidence in mammals has indicated that lipid metabolism obeys a circadian clock (Alila-Johansson et al., 2004; Bertolucci et al., 2008; Bitman et al., 1990; Eckel-Mahan et al., 2012; Escobar et al., 1998; Mauvoisin et al., 2014; Panda et al., 2002 ; Piccione et al., 2003; Robles et al., 2014; Storch et al., 2002). In addition, the results of several research works have suggested that LD and feeding cycles are powerful synchronizers of central (brain) and peripheral (liver) biological clocks. For the latter two, there is increasing awareness that peripheral clocks are highly susceptible, to the extent that the working pattern decouples from the central master clock (SCN) to obey other synchronizing cues (Damiola et al., 2000; Hara et al., 2001; Schilber, 2009; Stokkan et al., 2001), and becomes tissue-specific for particular physiological processes (Betancor et al., 2014). According to several reports (Eckel-Mahan et al. 2013; Pendergast et al. 2013; Sánchez-Vázquez et al. 2001), the liver is the most sensitive tissue to food intake, composition and timing, so much so that it drastically alters its daily rhythmicity (with regard to clock gene expression). Similarly according to our results, the rhythmicity of *lxr* shifted in the MD group. This nuclear receptor is involved in lipogenic gene regulation and is responsive to oxysterols, which are oxidized derivatives of cholesterol and are involved in cholesterol homeostasis (Schroepfer, 2000). This gene also plays an important role in adipocyte differentiation through the activation of *pparγ*, *srebp* and *fas* (Seo et al., 2004). These data suggest that the daily rhythm expression of *lxr* may vary depending on physiological functions other than lipogenesis; i.e. the oxidative state that fluctuates with feeding time (ML, MD or RD). Yet for the other studied genes, feeding time was found to have barely any influence. The results reveal that most of the hepatic genes analyzed in zebra fish adjusted to an LD synchronizer, with a nocturnal acrophase for lipogenic and lipolytic genes at around Ø = ZT18:00 h and

Ø = ZT20:30 h, respectively. Similarly, Szántóvá et al. (2011) in Wistar rats, and Paredes et al. (2014) in gilthead seabream observed that light best controlled hepatic lipid metabolic gene expression. Sigurgeirsson et al. (2013) also reported up-regulation in lipogenic genes during the dark period, which is indicative of a relationship with sleep-wake dynamics. These data suggest that the activities of the studied genes are under the direct control of a master LEO central clock. It does not escape our notice that anabolic and catabolic activities operate at different times of the day, and accomplish their respective metabolic roles efficiently (Bolliet et al., 2000; Lal et al., 1999). We can state that lipogenesis and lipolysis rhythms did not vary with feeding time; consequently, these results allowed us to suggest that feeding in the lipogenesis phase may lead to fish fattening. In line with this, Garaulet & Gomez-Abellan (2014) also stated that timing of food intake in human beings plays a crucial role in fat storage because adipose tissue genes are displayed in a specific temporal order by either accumulating or mobilizing fat. Thus, the distribution of food energy in fat stores depends on the time of food intake.

In summary, this article describes for the first time daily rhythms of expression of lipid metabolic genes in zebra fish liver and their synchronization to LD and feeding cycles. Further investigation to comprehend the time-line operating mechanism from RNA expression to the absorption, transport and metabolism of lipids is warranted. Besides, further research into circadian tissue-specific functioning and its connection with the master central clock will help us understand the intermingled network mechanism of the circadian system as a whole. Nevertheless, our results provide significant insight into the molecular metabolic mechanisms by highlighting evident light control with high nocturnal values for most of the investigated genes. Interestingly, feeding time barely influenced the daily rhythms of gene expression. This knowledge also provides a practical approach to optimize lipid metabolism when manipulating feeding and light cycles in fish farming.

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DECLARATION OF INTEREST

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