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Interaction Between Photoperiod and Sex on Hepatic Lipid Homeostasis in Rats Fed an Obesogenic Diet

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ABSTRACT

Seasonal changes in day length as well as sex are critical factors influencing metabolic processes. However, their combined impact on liver lipid metabolism under obesogenic conditions remains unclear. This study aims to investigate the interaction between sex and photoperiod on liver lipid homeostasis in obese Fischer 344 (F344) rats, with a special emphasis on the underlying molecular mechanisms. To this aim, male and female rats were fed with a cafeteria diet (CAF) for 11 weeks and exposed to either long (L18, 18 h of light/day) or short (L6, 6 h of light/day) photoperiods for the last 8 weeks. Liver histological analysis, hepatic damage markers, plasmatic and hepatic lipid profiles as well as hepatic gene expression related to lipid metabolism were performed. Results indicate that males exposed to the L18 photoperiod developed hepatic macro-steatosis and exacerbated hepatic lipid accumulation, leading to increased liver weight and total hepatic lipids compared to their L6 photoperiod counterparts. At the molecular level, males under the L18 photoperiod, showed downregulation of the lipid transporter *Fabp4* and upregulation of β-oxidation related genes. On the other hand, female rats exhibited a healthier lipid profile compared to males when exposed to both L6 and L18 photoperiods, and a lower degree of steatosis. Gene analysis revealed that the lipid transporter *Mtp* expression was significantly higher in females under the L18 photoperiod. In conclusion, photoperiod and sex influence hepatic lipid metabolism in rats fed an obesogenic diet, highlighting the importance of considering these factors in understanding and addressing metabolic disorders and reinforcing the concept of precision medicine.

1 | Introduction

Changes in day length (photoperiods) throughout the year are critical environmental cues that regulate circannual biological processes in many species, especially in mammals [1, 2]. These seasonal rhythms are essential for maintaining physiological homeostasis, helping organisms to anticipate and adapt to predictable environmental changes such as temperature, food

Abbreviations: *Abca-1*, ATP binding cassette subfamily A member 1; *Acaca*, acetyl-CoA carboxylase alpha; ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; CAF, cafeteria diet; *Cpt-1a*, carnitine palmitoyl transferase type 1A; *Dgat-2*, diacylglycerol *O*-acyltransferase 2; F344, Fischer 344; *Fabp-4*, fatty acid-binding protein 4; *Fasn*, fatty acid synthase; HDL-C, high-density lipoproteins; HFD, high fat diet; *Hmg-CoA reductase*, 3-hydroxy-3-methyl-glutaryl coenzyme-A reductase; H-PL, hepatic phospholipids; H-TAG, hepatic triglycerides; H-TC, hepatic total cholesterol; IL-1β, interleukin-1β; IL-6, interleukin-6; L6, short photoperiod; L12, standard photoperiod; L18, long photoperiod; LDL-C, low-density lipoprotein; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease; MetS, metabolic syndrome; *Mtp*, microsomal triglyceride transfer protein; NAFLD, non-alcoholic fatty liver disease; NEFA, non-esterified fatty acids; *Ppar-α*, peroxisome proliferator-activated receptor alpha; *Ppia*, peptidylprolyl isomerase A; *Scarb1*, scavenger receptor class B member 1; *Srebp-1c*, sterol regulatory element-binding protein 1; T3, triiodothyronine; TAG, triglycerides; TC, total cholesterol; TNF-α, tumor necrosis factor alpha; VLDL, very-low density lipoproteins.

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availability, and reproductive cycles [3]. Photoperiods influence metabolic and behavioral adaptations, primarily regulated by melatonin production, secreted by the pineal gland during dark [2]. Several studies have shown the differential effect of photoperiods on lipid metabolism in male animals. For instance, photoperiods with more than 12 h of light per day have been shown to induce an anabolic state, promoting lipid storage, while shorter photoperiods favor a catabolic state, characterized by lipid mobilization and increased energy expenditure [4]. Specifically, it has been shown that long photoperiod exposure increases body fat mass through attenuation of brown adipose tissue activity, without affecting the daily food intake of a chow diet in male rats [5]. Moreover, the levels of plasmatic and hepatic total cholesterol were higher in rats exposed to long photoperiods when compared to short photoperiods [6]. In addition, Mariné-Casadó et al. [7] demonstrated that male Fischer 344 (F344) rats exposed to long photoperiods exhibited higher blood glucose levels and significant changes in lipid metabolism parameters in the blood, liver, and skeletal muscles when compared to rats under short photoperiod conditions. In high fat diet (HFD)-fed male rats, these photoperiodic adaptations were disrupted by the dysregulation of hormone profiles, leading to metabolic alterations, such as dyslipidemia and changes in glucose metabolism [8].

On the other hand, several studies have reported that HFD alters corticosterone levels, decreases energy expenditure, and affects the expression of hepatic genes involved in lipid transport and storage, promoting fat accumulation and reducing lipid flux through hepatic metabolic pathways [9]. In obesity models, the hepatic gene expression of the microsomal triglyceride transfer protein (*Mtp*), which is in charge of assembling and secreting very low-density lipoproteins (VLDL), is upregulated, contributing to a dyslipidemia status by increasing the secretion of triglyceride-rich lipoproteins [9]. In contrast, upregulation of the fatty acid binding protein 4 (*Fabp4*) in the liver has been correlated with fat accumulation and activation of preadipogenic genes [10, 11]. This fat accumulation can lead to enhanced lipid uptake and storage in hepatocytes, thereby exacerbating non-alcoholic fatty liver disease (NAFLD) [11]. In addition, hepatic lipogenesis is increased while β-oxidation is decreased as a result of the high flux of fatty acids in HFD [12, 13]. This leads to the development of metabolic dysfunction-associated steatotic liver disease (MASLD) [14], a common liver condition characterized by hepatic lipid accumulation due to increased fatty acid uptake, increased de novo lipogenesis, and impaired β-oxidation, closely associated with metabolic risk factors [15, 16]. Moreover, this lipid imbalance promotes oxidative stress and inflammation, contributing to liver injury and disease progression [17]. Metabolic dysfunction-associated steatohepatitis (MASH) is a more advanced stage in the development of MASLD and is characterized by severe hepatic steatosis, lobular inflammation, and globular degeneration [18].

Male and female rats showed differences between lipid homeostasis in the liver and the development of MASLD. In this regard, female rats fed a HFD supplemented with liquid fructose for 12 weeks developed hepatic steatosis, whereas male rats did not exhibit such lipid accumulation [19]. Additionally, male rats displayed higher levels of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), or interleukin-6 and 1β (IL-6 and IL-1β), compared to females, indicating different mechanisms in liver disease progression when fed a HFD [20]. In addition, female rats have shown greater resistance to diet-induced hepatic steatosis and better maintenance of lipid homeostasis compared to males [21, 22]. Moreover, the expression of key enzymes involved in lipogenesis and fatty acid oxidation demonstrates significant sexual dimorphism, with females typically exhibiting higher rates of hepatic fatty acid oxidation compared to males [23, 24]. Cholesterol metabolism also reveals notable sex-specific variations, with female rats displaying higher bile acid synthesis and excretion rates, suggesting a potential protective mechanism against cholesterol accumulation when fed a HFD [25].

While these effects of photoperiods and sex on liver lipid homeostasis have been studied, their interaction and the underlying molecular mechanisms remain poorly understood. Hence, we hypothesize that female rats under obesogenic conditions compared to males will modulate the liver lipid homeostasis differently depending on the day length of exposure. Therefore, this study aimed to investigate the interaction of sex and photoperiod on the disturbance of liver lipid homeostasis induced by an obesogenic diet, with a particular focus on the associated molecular mechanisms.

2 | Materials and Methods

2.1 | Experimental Procedure in Rats

Forty male and female ($n = 20$) 12-week-old Fischer 344 (F344) rats were acquired from Charles Rivers Laboratories (Barcelona, Spain) (Figure 1). Animals were housed in pairs at 22°C and 50% humidity under a reverse light cycle (lights off at 8 a.m.) and standard photoperiod conditions (L12, 12 h light/day). After 1 week of adaptation to a standard diet and L12 photoperiod, rats were fed a cafeteria (CAF) diet for 11 weeks with tap water provided ad libitum. This diet consists of high-fat and high-caloric human foods such as bacon, ensaïmada (pastry), biscuits with pâté and cheese, carrots, standard chow and milk containing 22% sucrose (w/v), and the calorie breakdown is 14% proteins, 51% carbohydrates, and 35% fat. Animals were daily fed with freshly prepared CAF 1 h after the lights were turned off. After a 3-week diet adaptation period under L12 conditions, both female and male animals were divided into two different groups ($n = 10$/group) for the last 8 weeks of the experiment: (1) rats exposed to long photoperiod (L18, 18 h light/day) and (2) rats exposed to short photoperiod (L6, 6 h light/day).

On the last day of experiment, animals were fasted for 3 h upon lights were turned off (8 a.m.) and then sacrificed by decapitation. Blood was collected in heparinized tubes and then centrifuged at 1200 *g* for 15 min to collect the plasma. Liver was dissected, weighted and froze in liquid nitrogen for further analysis. For histological analysis, one lobe of liver was fixed in 4% formaldehyde for 24 h and then transferred to 70% ethanol to later undergo dehydration steps with higher concentrations of ethanol.

Animal experiments were conducted in accordance with the European Communities Council Directive (86/609/EEC) and approved by the Animal Ethics Review Committee for Animal Experimentation of the Universitat Rovira i Virgili and Generalitat de Catalunya (permission number 11610).

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2.2 | Biochemical Parameters in Plasma

In plasma, non-esterified fatty acids (NEFA) were analyzed using a FujiFilm commercial kit (WAKO, Neuss, Germany); total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TAG) were analyzed in an automatic analyzer Cobas Mira Plus Autoanalyzer (Roche Diagnostics, Spain) using commercial kits (Spinreact SA, Spain). Hepatic damage markers (alanine aminotransferase [ALT], aspartate aminotransferase [AST], and bilirubin) and bile acids were analyzed using commercial kits (from QCA, Spain, to analyze ALT and AST; and from Spinreact SA, Spain, for bile acids and bilirubin analysis).

2.3 | Liver Parameters

For liver total lipid content, 50 mg of tissue was homogenized with 200 μL of chloroform, 200 μL of methanol, and 100 μL of water by Tissue Lyser LT (Qiagen, Madrid, Spain), and centrifuged for 10 min at 3000 *g* and 4°C. The lower phase was transferred to a new Eppendorf tube and let the chloroform evaporate overnight at room temperature. Eppendorf tubes with lipid pellets were weighed and total lipid content was calculated.

For lipid parameters determinations, lipids were dissolved and homogenized using 2% Triton X-100 and with the lipid extraction, commercial kits were used to measure levels of total hepatic triglycerides (H-TAG), total hepatic cholesterol (H-TC) (QCA, Spain), and hepatic phospholipids (H-PL) (Spinreact SA, Spain).

2.4 | Histopathological Analysis

A piece of liver ($n = 8$) was sent for histopathology (Eldine facilities, Tarragona, Spain). One lobe of liver was fixed in 4% formaldehyde for 24 h to later undergo dehydration steps with ethanol (70%, 96% and 100%) in addition to xylol/dimethyl benzene, and paraffin infiltration at 52°C (Citaldel 2000; Thermo Scientific, Madrid, Spain). Stained slides were analyzed by a pathologist blinded to experimental groups to measure the steatosis degree, percentage of micro- and macro-steatosis, and macro-steatosis localization, lipogranuloma, microgranuloma, portal chronic inflammation, sinusoidal dilatation, fibrosis degree and perivenular inflammation. Scores used for each parameter are detailed in Table 1.

2.5 | RNA Extraction

Total RNA was isolated from liver (20–30 mg) with Trizol reagent (Thermo Fisher, Madrid, Spain) and homogenized by Tissue Lyser LT (Qiagen, Madrid, Spain). After centrifuging

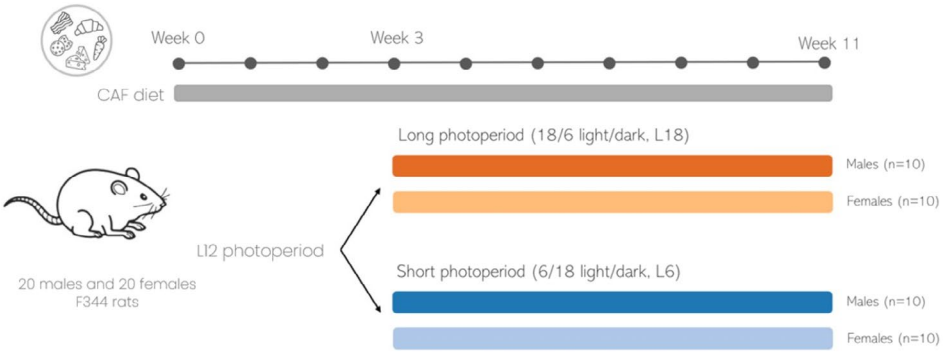

FIGURE 1 | Experimental design. Twenty male and 20 female F344 rats were fed for 11 weeks with CAF diet and exposed to L18 and L6 photoperiods for the last 8 weeks of the experiment. CAF, cafeteria; F344, Fischer 344; L6, short photoperiod of 6 h of light per day; L12, standard photoperiod of 12 h of light per day; L18, long photoperiod of 18 h of light per day.

TABLE 1 | Scores given to the analysis of steatosis degree, portal chronic inflammation, sinusoidal dilatation, fibrosis degree and perivenular inflammation.

Score	0	1	2	3	4
Steatosis degree	< 5%	5%–33%	33%–66%	> 66%	
Chronic portal inflammation	Absent	Mild	Moderate	Marked	
Sinusoidal dilatation	Absent	Mild	Moderate		
Degree of fibrosis	Absent	Portal fibrous expansion	Incomplete porto-portal or porto-centrilobular bridges	Complete porto-portal or porto-centrilobular bridges	Cirrhosis
Perivenular inflammation	Absent	Mild	Moderate	Marked	

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for 10 min at 12 000 *g* and 4°C, the homogenate was placed in a new Eppendorf and added 120 μL of chloroform. After centrifuging it for 15 min at 12 000 *g* at 4°C, two phases were separated, and the aqueous phase was transferred into a new Eppendorf tube with 300 μL of isopropanol. After an overnight incubation at −20°C, samples were centrifuged at 12 000 *g* for 10 min at 4°C, and the supernatant was discarded. The pellet was cleaned twice with 500 μL of ethanol and centrifuged for 5 min (8000 *g* at 4°C). The supernatant was discarded and allowed to let ethanol evaporate at room temperature. The pellet was resuspended with 60 μL of nuclease-free water (Thermo Fisher, Madrid, Spain).

2.6 | Gene Expression Analysis

RNA was reverse transcribed using a high-capacity complementary DNA reverse-transcription kit (Thermo Fisher, Madrid, Spain). Quantitative real-time polymerase chain reaction (qPCR) was performed using SYBR Green Master Mix on 384-well plates of the QuantStudio 5 Real-Time PCR System (Thermo Fisher, Madrid, Spain). The thermal-cycle program used in all qPCRs was 30 s at 90°C, 40 cycles of 15 s at 95°C and 1 min at 60°C. The analyzed liver genes were normalized by the peptidylprolyl isomerase A (*Ppia*) gene, used as a housekeeping gene. Primers used for each gene were obtained from Biomers (Ulm, Germany) and are shown in Table S1. Cycle threshold (Ct) values were recorded, normalized to housekeeping gene expression and transformed to relative mRNA level values using the $2^{-\Delta\Delta Ct}$ method reported by Livak and Schmittgen [26]. The relative mRNA levels of all groups were referred to the group of males exposed to long photoperiod conditions.

2.7 | Western Blot Analysis

Liver protein extracts (50 μg) denatured and loaded into 15% or 12% (for FABP-4 or MTP protein, respectively) SDS-polyacrylamide gels (TGX FastCast Acrylamide Kit, Bio-Rad, Madrid, Spain) and run at 100 V for 90 min. Afterwards, gels were transferred onto a PVDF membrane using the Trans-Blot Transfer System (Bio-Rad, Madrid, Spain). Efficient protein transfer was assessed by Ponceau-S stain. Membranes were incubated for 1 h at room temperature in blocking buffer (5% non-fat dry milk diluted in 0.2% TBS-Tween) and then incubated overnight at 4°C with the polyclonal primary antibodies at a 1:1000 dilution of rabbit-anti-MTP, rabbit-anti-FABP4 and mouse-β-actin.

Thereafter, after washing three times with 0.2% TBS-Tween, membranes were incubated for 1 h with the peroxidase-conjugated secondary antibodies goat anti-mouse IgG and donkey anti-rabbit IgG (Amersham, Cytiva, Barcelona, Spain) at a 1:2000 dilution. After three more washes, the protein was detected with the chemiluminescent reagent ECL Select Western Blotting Detection Reagent (GE Healthcare, Barcelona, Spain). Protein levels were quantified using Image Lab 6.1 Software (Bio-Rad, Madrid, Spain) and normalized to β-actin (A2228) (Sigma–Aldrich) protein levels.

2.8 | Statistical Analysis

Results are presented as box plots with whiskers showing minimum to maximum values, median and interquartile range, using GraphPad Prism software (V9; Dotmatics).

Data was first assessed for normality by the Shapiro–Wilk test and for homoscedasticity by Levene's test. For parametric data, statistical comparisons between more than two groups were performed using two-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test for multiple comparisons. For non-parametric data, a two-way non-parametric ANOVA based on the Aligned Rank Transform (ART) was performed using the ARTool package in R [27]. In cases where significant main or interaction effects were observed, post hoc comparisons were performed using the ART-C procedure with Tukey adjustment, using the emmeans package in R [28], to examine photoperiod and sex effects.

Two-sided p values <0.05 were considered statistically significant, while p values between 0.05 and 0.1 were considered as trends. Statistical analyses were conducted using GraphPad Prism software (V9; Dotmatics) and RStudio (2024.04.1).

3 | Results

3.1 | CAF-Fed Female Rats Had a Better Plasmatic Lipid Profile Than Males in Both Long and Short Photoperiods

Plasma lipid parameters showed no significant differences between photoperiods, except for NEFA, but exhibited clear sex-specific variations in all the evaluated parameters (Table 2). Females displayed lower levels of NEFA compared to males in both long (L18) and short (L6) photoperiods. Furthermore, females demonstrated a more favorable lipid profile characterized by HDL-C levels and lower LDL-C and TAG levels compared to males in both photoperiods. Otherwise, bile acid levels displayed an interaction between sex and photoperiod. Males exposed to L18 conditions had lower bile acid levels compared to those in L6. Additionally, a strong sex effect was observed in L18, with females showing significantly higher bile acid levels than males.

3.2 | Exposure to Long Photoperiod Conditions Led to Higher Degree of Hepatic Steatosis and Was More Severe in Males

Histological analysis of hepatic steatosis revealed significant differences between sexes and photoperiods (Figure 2; Table 3).

Although at a moderate level, rats exposed to long photoperiod had the highest degree of steatosis among the male groups, with a markedly reduced percentage of microsteatosis and an increased macrosteatosis. In the case of females, those exposed to short photoperiod presented the lowest degree of hepatic steatosis, which was either absent or mild, and exhibited increased microsteatosis and decreased macrosteatosis. Female and male rats exposed to long photoperiod and short photoperiod respectively, showed a

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TABLE 2 | Effect of sex and photoperiod on plasmatic lipid parameters in male and female F344 rats fed with CAF diet for 11 weeks and exposed to L18 or L6 photoperiods for the last 8 weeks.

	L18 photoperiod		L6 photoperiod		
	Males	**Females**	**Males**	**Females**	**Effect**
NEFA (mg/dL)	50.37 (45.85–55.55)	32.90 (28.58–43.93)***	43.10 (39.53–48.62)	30.90 (27.84–39.21)*	S; P
TC (mg/dL)	103.35 (96.50–119.88)	146.70 (134.43–159.15)***	122.60 (103.20–149.50)	148.20 (136.00–153.40)	S
HDL-C (mg/dL)	55.79 (53.04–59.99)	79.31 (70.80–81.05)***	51.90 (46.13–55.75)	77.11 (72.34–82.11)***	S
LDL-C (mg/dL)	4.65 (4.03–5.08)	3.00 (2.78–3.80)*	3.30 (2.85–5.25)	3.10 (2.78–3.53)	S
TAG (mg/dL)	298.90 (225.15–375.83)	168.15 (122.63–294.60)	390.20 (281.90–459.65)	132.20 (120.50–164.85)****	S; S × P
Bile acids (mg/dL)	4.04 (4.04–4.88)	11.80 (7.20–14.26)***	8.93 (6.70–11.16)$	9.69 (5.87–13.36)	S; S × P

Note: Data are expressed as median (quartile 1 [Q1]–quartile 3 [Q3]) ($n = 10$). P, photoperiod effect, S, sex effect; S × P, interaction between sex and photoperiod, using two-way ANOVA; *Indicates significant differences (*$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$) between sexes in each photoperiod, and "$" indicates significant differences ($^{\$}p < 0.05$) between photoperiods in each sex, using two-way ANOVA and Tukey as post hoc comparisons.
Abbreviations: CAF, cafeteria diet; F344, Fischer 344; L6, short photoperiod of 6 h of light per day; L18, long photoperiod of 18 h of light per day; NEFA, non-esterified free fatty acids; TAG, triglycerides; TC, total cholesterol.

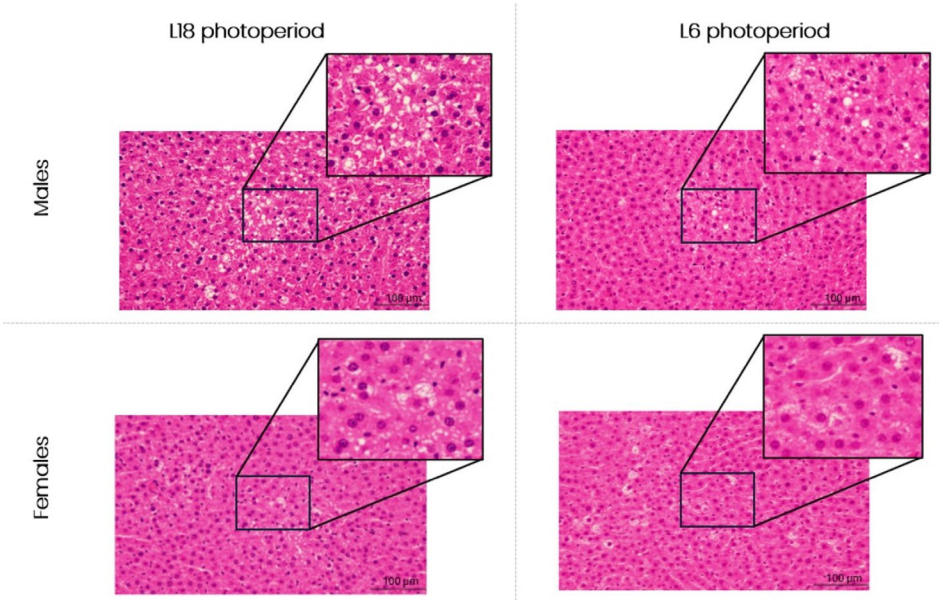

FIGURE 2 | Representative histological sections after eosin and hematoxylin stain of liver from male and female F344 rats exposed for 8 weeks to L18 or L6 photoperiods and fed with a CAF diet for 11 weeks. Pictures were taken under the magnification of 20×. CAF, cafeteria; F344, Fischer 344; L6, short photoperiod of 6 h of light per day; L18, long photoperiod of 18 h of light per day.

mild or absent degree of steatosis and a high percentage of microsteatosis. Altogether, in long photoperiod conditions the degree of hepatic steatosis with the presence of macrosteatosis is higher than in short photoperiod conditions, mainly in male rats. The distribution pattern of macrosteatosis varied across groups: females in both photoperiods and males in L6 conditions presented an azonal distribution, whereas males in L18 conditions displayed a distinct panacinar and centrilobular localization. Only one male rat under L18 conditions presented lipogranuloma, while the rest of the animals of the study presented microgranulomas.

No differences were observed in chronic portal inflammation, which was mild to moderate in all groups. In addition, all groups exhibited a mild degree of perivenular inflammation, indicating that a low-level inflammatory response was present in the liver tissue across all experimental conditions.

Sinusoidal dilatation was higher in female rats independently of photoperiod, being absent in males under L18. Moreover, female rats exposed to L6 photoperiod had higher sinusoidal dilatation than females exposed to L18 photoperiod.

Histological evaluation revealed distinctive patterns of hepatic fibrosis among the studied groups. Thus, males and females under L6 conditions as well as males under L18 photoperiod exhibited an absence of fibrosis. However, females subjected to the L18 photoperiod presented portal fibrous expansion, representing the initial stage in the development of hepatic fibrosis.

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TABLE 3 | Liver histopathological analysis of males and females F344 rats fed with CAF diet for 11 weeks and exposed to L18 or L6 photoperiods for the last 8 weeks.

	L18 photoperiod		L6 photoperiod		
	Males	**Females**	**Males**	**Females**	**Effect**
Steatosis					
Degree (0–3, in severity)	2 (1–2)	1 (0–1)	0 (0–1)	0.5 (0–1)	
% microsteatosis (0–100)	66%	86%	88%	94%	S, P, S×P
% macrosteatosis (0–100)	34%	14%	12%	6%	S, P, S×P
Localization of macrosteatosis	6/8 centrilobular 2/8 panacinar	8/8 azonal	7/8 azonal 1/8 centrilobular	6/6 azonal	
Lipogranuloma (No. of samples)	1/8	0/8	0/8	0/8	
Microgranuloma (No. of samples)	8/8	7/8	7/8	7/8	
Chronic portal inflammation (0–3, in severity)	0 (0–0.75)	1 (1–1)	0.5 (0–1)	0.5 (0–1)	
Sinusoidal dilatation (0–2, in severity)	0 (0–1)	1 (1–1)	1 (1–1)	1.5 (1–2.75)	
Degree of fibrosis (0–4, in severity)	0 (0–0)	1 (1–1)	0.5 (0–1)	0.5 (0–1)	
Perivenular inflammation (0–3, in severity)	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–2)	

Note: Data are expressed as median (quartile 1 [Q1]–quartile 3 [Q3]) ($n = 8$). P, photoperiod effect; S, sex effect; S×P, interaction between sex and photoperiod using two-way ANOVA.
Abbreviations: CAF, cafeteria diet; F344, Fischer 344; L6, short photoperiod of 6 h of light per day; L18, long photoperiod of 18 h of light per day.

3.3 | Liver Damage Induced by Cafeteria Diet Feeding Is Lower in Males Independently of Photoperiods

Liver damage markers in plasma showed distinct patterns between sexes and photoperiods. In this regard, while AST levels remained similar across sexes and photoperiods, males exposed to L6 photoperiod exhibited higher ALT levels compared to females under the same light conditions. In addition, although not statistically significant, ALT levels were also higher in males exposed to L18 (Figure 3A,B). Consistently, the AST/ALT ratio was higher in females compared to males in both photoperiods (Figure 3C).

Bilirubin maintained similar levels between males and females in L18 conditions. In contrast, females showed lower levels than males under L6 conditions (Figure 3D).

3.4 | CAF-Fed Male Rats Exposed to Long Photoperiod Conditions Had Highest Hepatic Lipids Levels and Liver Weight

There was a sex and photoperiod effect in liver weight. In this sense, CAF-fed female rats had a lower percentage of liver weight than males when exposed to both photoperiods. Additionally, males exposed to L18 photoperiod showed a higher percentage of liver weight than males exposed to L6 photoperiod (Figure 4A). This agrees with a higher total liver lipid content in males at L18 conditions when compared to their counterparts at L6 and to females at the same L18 conditions (Figure 4B).

Consistently, there is a sex effect and an interaction between sex and photoperiod in H-TC and H-TAG (Figure 4C,D). Male rats exposed to L18 conditions showed a trend toward increased H-TC and H-TAG when compared to males at L6 and to female rats under the same L18 conditions. In addition, a photoperiod effect was observed in females for both H-TAG and H-PL levels being significantly lower in female rats exposed to L18 conditions. No effect was observed in male rats (Figure 4E).

3.5 | CAF-Fed Female Rats Under Short Photoperiod Conditions Had Reduced Levels of *Srebp-1C* Than Under Long Photoperiods and Increased Levels of *Dgat* Than Males in the Same Short Photoperiod Exposure

Relative mRNA hepatic expression level of fatty acid synthase (*Fasn*), acetyl-CoA carboxylase alpha (*Acaca*), sterol regulatory element-binding protein 1c (*Srebp-1C*), and diacylglycerol *O*-acyltransferase (*Dgat*) was analyzed in order to investigate the effects of sex and photoperiod conditions on hepatic lipogenesis. *Fasn* relative expression was significantly affected by sex while no impact of photoperiods was observed (Figure 5A). In the case of *Acaca* relative expression, neither sex nor photoperiod effects were found (Figure 5B). However, a photoperiod effect was noticeable in female rats when studying the relative gene expression of *Srebp-1C*. In this sense, CAF-fed female rats under short photoperiod had decreased mRNA levels of this transcription factor than those exposed to long photoperiod conditions (Figure 5C). Interestingly, there was

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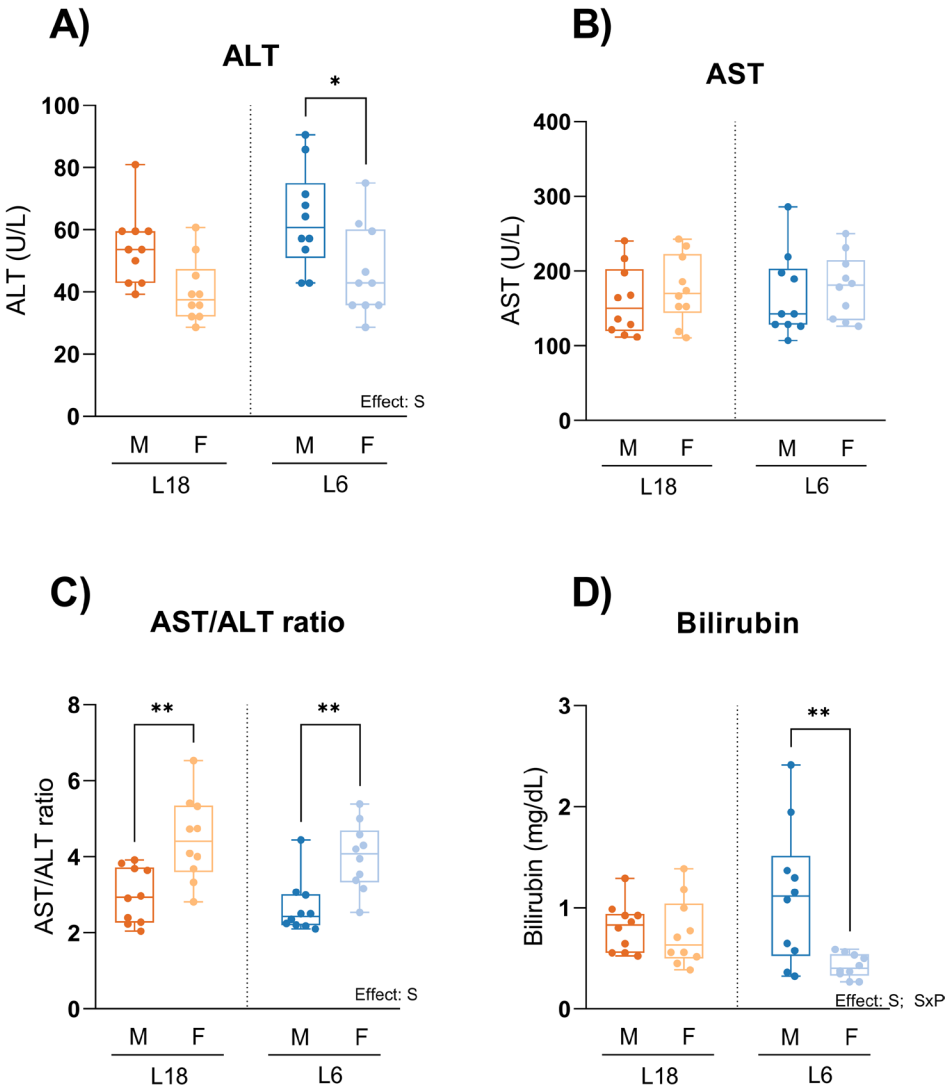

FIGURE 3 | Effect of sex and photoperiod on hepatic damage markers in plasma. ALT (A), AST (B), AST/ALT ratio (C) and bilirubin (D) in male and female F344 rats fed with CAF diet for 11 weeks and exposed to L18 or L6 photoperiods for the last 8 weeks. Data are expressed as minimum to maximum values, median and interquartile range ($n = 10$). P, photoperiod effect; S, sex effect; S × P, interaction between sex and photoperiod assessed using two-way ANOVA; *Indicates significant differences (*$p < 0.05$, **$p < 0.01$) between groups using two-way ANOVA followed by Tukey post hoc test. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAF, cafeteria; F, females; F344, Fischer 344; L6, short photoperiod of 6 h of light per day; L18, long photoperiod of 18 h of light per day; M, males.

a sex effect in *Dgat* and its gene expression was upregulated in females when compared to their male counterparts under short photoperiod regimens (Figure 5D).

3.6 | CAF-Fed Male Rats Had Decreased Gene Expression of Hepatic β-Oxidation Gens When Exposed to Short Photoperiod Conditions

β-Oxidation in the liver was evaluated by the analysis of the relative mRNA expression levels of both carnitine palmitoyltransferase 1 alpha (*Cpt-1α*) and peroxisome proliferator-activated receptor alpha (*Ppar-α*). In CAF-fed male rats, there was a photoperiod effect for the relative expression of hepatic *Cpt-1α* which was downregulated under L6 conditions. In contrast, no significant photoperiod effect was observed for this gene in females. However, a tendency toward higher *Cpt-1α* expression was observed in females when compared to males under L6 conditions (Figure 6A). Moreover, an interaction effect between sex and photoperiod was observed in the hepatic gene expression of *Ppar-α*. Specifically, female rats had a lower expression of *Ppar-α* compared to males when exposed to L18 conditions, while the opposite was observed in L6 conditions. Additionally, a photoperiod effect for *Ppar-α* hepatic expression levels was observed in males, being lower when exposed to L6 conditions (Figure 6B).

3.7 | CAF-Fed Female Rats Had Increased Gene Expression of the Hepatic Lipid Transporter *Mtp* Under Long Photoperiod Conditions, While Male Rats Had Increased Gene Expression of *Fabp-4* When Exposed to Short Photoperiod Conditions

To elucidate the effects in hepatic lipid transport, the gene expression of *Mtp* and *Fabp-4* in liver was examined.

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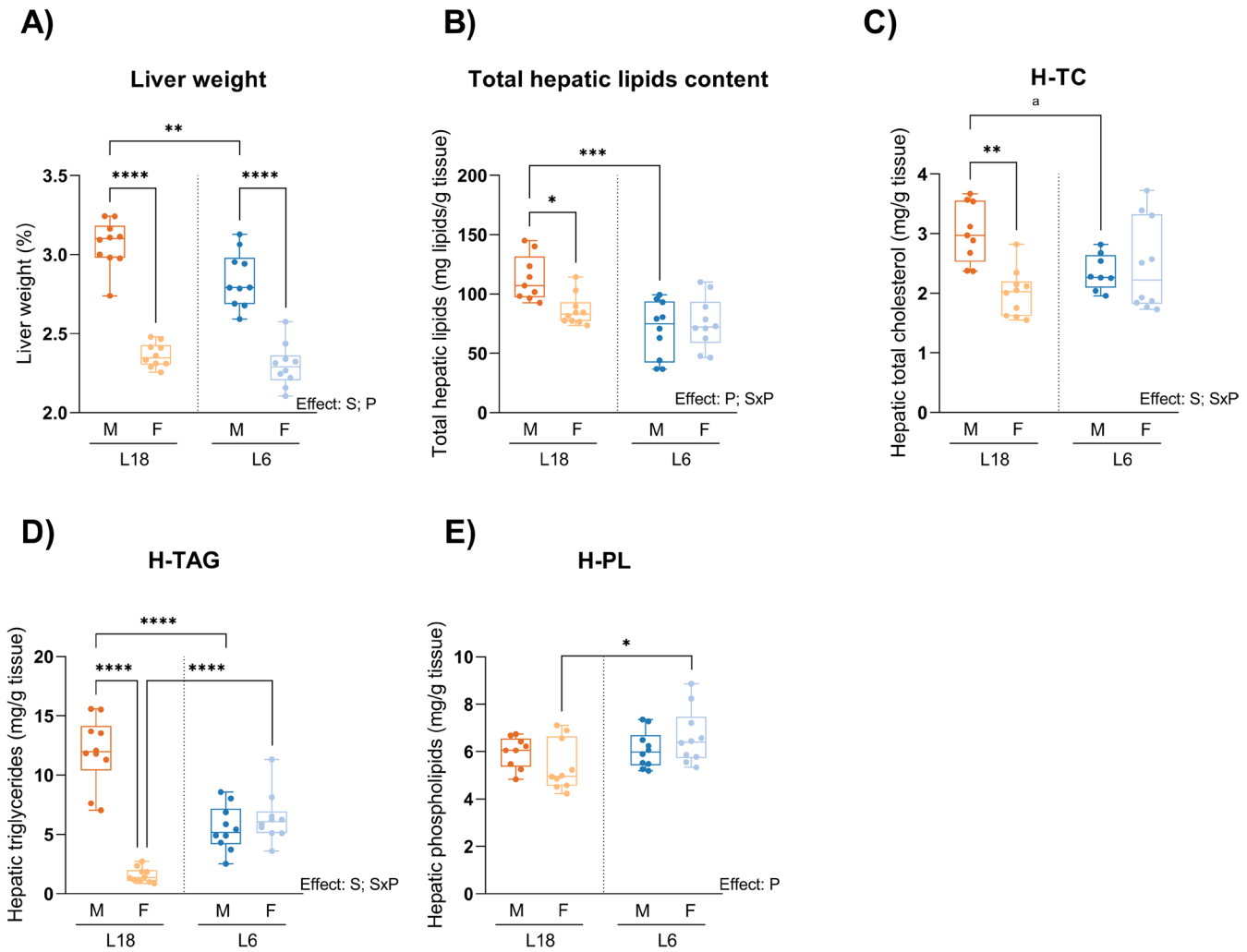

FIGURE 4 | Effect of sex and photoperiod on hepatic lipid parameters. Liver weight (A), total hepatic lipid content (B), hepatic total cholesterol (C), hepatic triglycerides (D), hepatic phospholipids (E) in male and female F344 rats fed with CAF diet for 11 weeks and exposed to L18 or L6 photoperiods for the last 8 weeks. P, photoperiod effect; S, sex effect; S×P, interaction between sex and photoperiod assessed using two-way ANOVA. *Indicates significant differences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$) and "a" indicates a trend ($p < 0.1$) between groups using two-way ANOVA followed by Tukey post hoc test. CAF, cafeteria; F, females; F344, Fischer 344; H-PL, hepatic phospholipids; H-TAG, hepatic triglycerides; H-TC, hepatic total cholesterol; L6, short photoperiod of 6 h of light per day; L18, long photoperiod of 18 h of light per day; M, males.

Interestingly, substantial differences were observed in *Mtp* gene expression. Accordingly, CAF-fed female rats exposed to L18 conditions exhibited a remarkable higher *Mtp* expression. In this sense, a photoperiod effect was observed in females, with lower *Mtp* expression levels when exposed to L6 conditions. In addition, under L18 conditions, female rats had higher *Mtp* expression than males (Figure 7A). Notably, MTP protein expression was mainly affected by sex, corroborating the gene expression results. Higher protein expression levels were found in females compared with males exposed to the L18 photoperiod, while no differences between photoperiods were found (Figure 7B).

Moreover, photoperiod- and sex-dependent effects as well as an interaction between them were observed for *Fabp-4* expression. Thus, CAF-fed male rats exposed to L6 had higher *Fabp-4* expression than females under the same conditions and those males exposed to L18 (Figure 7C). However, the protein levels did not show significant differences between groups (Figure 7D).

3.8 | Hepatic mRNA Levels of *Hmg-CoA Reductase* Showed an Interaction Effect Between Sex and Photoperiods

To study the mechanisms that affect cholesterol metabolism, scavenger receptor class B member 1 (*Scarb1*), ATP binding cassette subfamily A member 1 (*Abca1*) and 3-hydroxy-3-methylglutaryl coenzyme A reductase (*Hmg-CoA reductase*) mRNA levels were evaluated. No significant differences in terms of sex or photoperiod changes were observed in *Scarb1* and *Abca1*, although an interaction effect between sex and photoperiod was observed for *Hmg-CoA reductase* (Figure 8).

4 | Discussion

Seasonal oscillations, which are mainly modulated by changes in photoperiods, have been reported to significantly influence lipid metabolism [7, 29], especially under obesity conditions [8]. In this sense, MASLD is a common complication in obesity,

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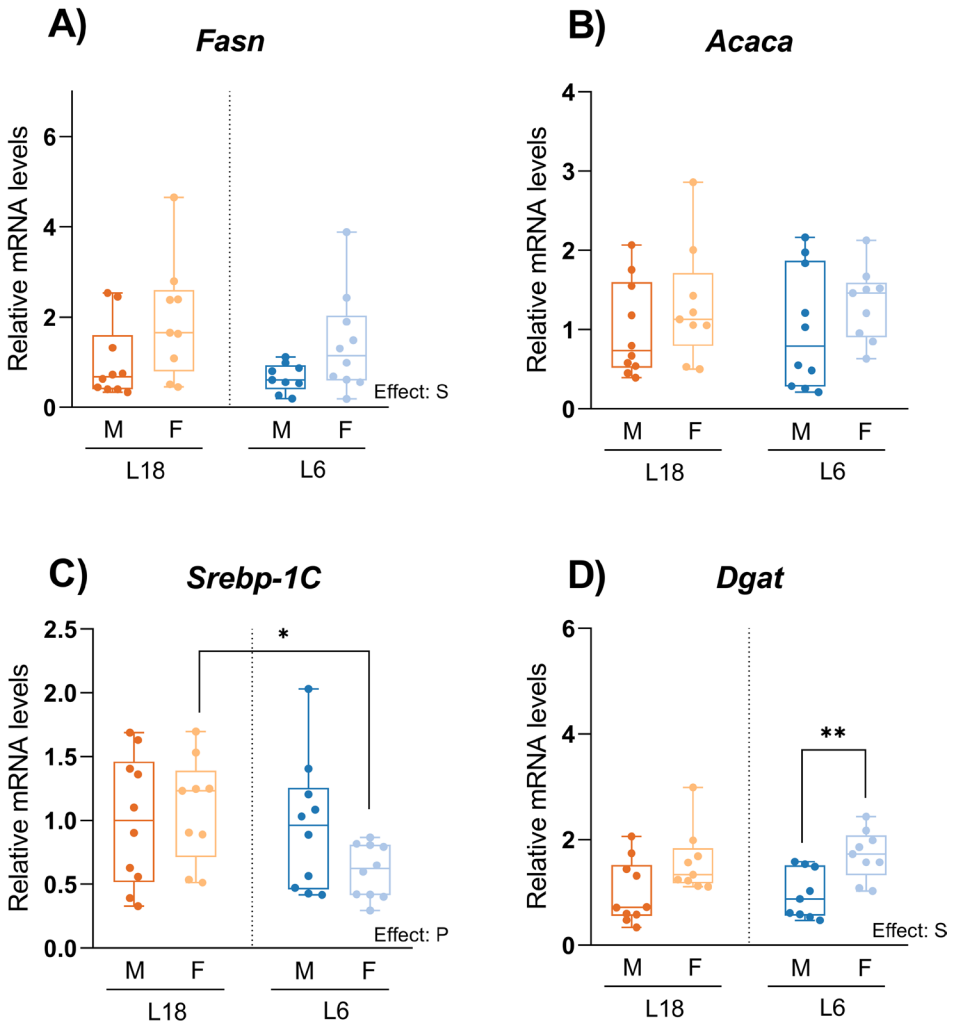

FIGURE 5 | Effect of sex and photoperiods on the relative gene expression of lipogenesis-related genes in the liver. *Fasn* (A), *Acaca* (B), *Srebp-1c* (C), and *Dgat* (D) relative hepatic mRNA levels in male and female F344 rats fed with CAF diet for 11 weeks and exposed to L18 or L6 photoperiods for the last 8 weeks. Data are expressed as minimum to maximum values, median and interquartile range ($n = 10$). P, photoperiod effect; S, sex effect assessed using two-way ANOVA for Srebp-1C, and two-way non-parametric ANOVA with ART transformation for *Fasn*, *Acaca* and *Dgat*. *Indicates significant differences (*$p < 0.05$ and ** $p < 0.01$) between groups using Tukey as post hoc test for Srebp-1C, and post hoc analysis with Tukey adjustment using emmeans package in R for *Fasn*, *Acaca* and Dgat. CAF, cafeteria; F344, Fischer 344; M, males; F, females; L18, long photoperiod of 18 h of light per *day*; L6, short photoperiod of 6 h of light per day; *Fasn*, Fatty acid synthase; *Acaca*, acetyl-CoA carboxylase alpha; Srebp-1c, sterol regulatory element-binding protein 1C; *Dgat*, diacylglycerol O-acyltransferase 1.

characterized by disruptions in hepatic lipid metabolism, leading to hepatic steatosis and potential progression to fibrosis or cirrhosis [12, 30]. There is a well-documented sexual dimorphism in hepatic lipid metabolism and in the development of MASLD, where females generally exhibit enhanced fatty acid oxidation and bile acid synthesis compared to males [23, 24, 31]. Although the effects of photoperiod and sex on liver lipid homeostasis have been evaluated in rats [6, 7, 32], there is a gap in knowledge of the interaction effect between photoperiods and sex as well as the molecular mechanisms implicated. Consequently, this study aimed to investigate the interplay between sex and photoperiods in the disruption of hepatic lipid homeostasis caused by an obesogenic diet, with a focus on the underlying molecular mechanisms. To this aim, male and female F344 rats were fed a cafeteria diet for 11 weeks and exposed to L18 and L6 photoperiods for the last 8 weeks, simulating seasonal light variations. Standard photoperiod (L12) was not used as it has been reported to induce similar hepatic lipid homeostasis to photoperiods with more than 12 h of light per day [4]. F344 rats are commonly used when changes related to photoperiods are under study, as they are a rat species sensitive to day length variations [33]. These rats exhibit physiological changes when they are exposed to different photoperiods, such as changes in lipid and glucose metabolism [7].

In this study, we observed that 11 weeks of CAF feeding induced a dyslipidemia state in both sexes. CAF is based on highly palatable and energy-rich foods that are commonly consumed by humans. This diet has been used in several studies with rats to induce obesity and metabolic syndrome (MetS) closely replicating human disease progression, including a dyslipidemia status [8, 34]. This dyslipidemia state caused by CAF feeding is characterized by higher levels of plasmatic TAG, TC and LDL-C [14, 35], along with higher hepatic lipid content when compared to rats fed a standard chow diet [14]. Indeed, in our study, plasma TAG and TC levels were markedly higher than those typically reported in

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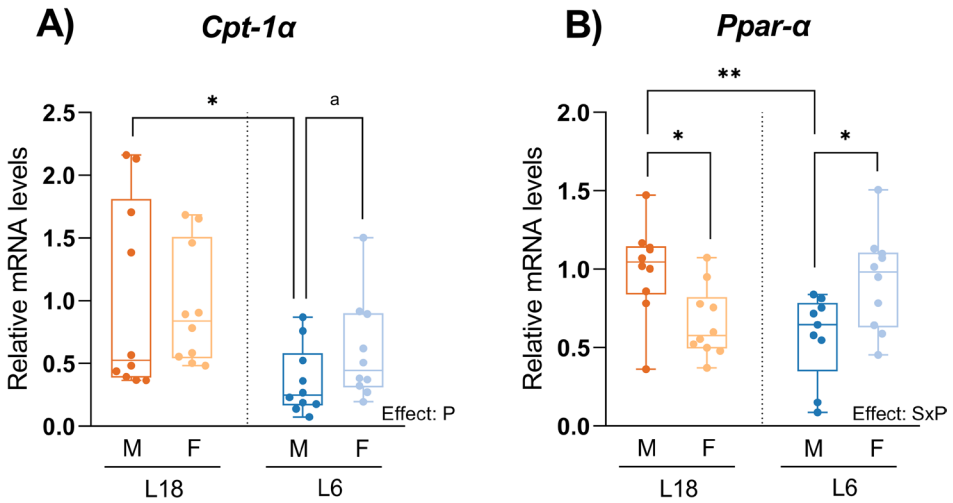

FIGURE 6 | Effect of sex and photoperiods on the relative gene expression of β-oxidation related genes in the liver. *Cpt-1α* (A) and *Ppar-α* (B) relative hepatic mRNA levels in male and female F344 rats fed with a CAF diet for 11 weeks and exposed to L18 or L6 photoperiods for the last 8 weeks. Data are expressed as minimum to maximum values, median and interquartile range ($n = 10$). P, photoperiod effect; S, sex effect; S × P, interaction between sex and photoperiod assessed using two-way ANOVA. *Indicates significant differences (*$p < 0.05$, **$p < 0.01$) and "a" indicates a trend ($p < 0.1$) between groups using two-way ANOVA followed by Tukey post hoc test. CAF, cafeteria; *Cpt-1α*, carnitine palmitoyltransferase I alpha; F, females; F344, Fischer 344; L6, short photoperiod of 6 h of light per day; L18, long photoperiod of 18 h of light per day; M, males; *Ppar-α*, peroxisome proliferator-activated receptor alpha.

standard chow-fed rats maintained under L12 photoperiod, further supporting the strong lipogenic and obesogenic potential of this diet [7, 36]. Our group has extensive experience working with CAF-induced obese rats. We have reported that 7 weeks of CAF feeding is sufficient to induce obesity status and MetS in rats, accompanied by dyslipidemia [7] as well as to increase plasmatic lipid levels in males exposed to different photoperiods [7, 8]. This is in accordance with the increased plasmatic levels of NEFA, TAG, and total cholesterol (TC) observed in this study.

The histological analysis of liver samples revealed a strong correlation between lipid accumulation patterns in the liver and the biochemical lipid profile, particularly in males exposed to L18 conditions. This group presented the highest degree of hepatic steatosis compared to the other groups. Predominance of steatosis was observed in the centrilobular zone, which aligns with previous studies [37, 38]. The differences observed in this work between sexes, are in contradiction with two parallel studies that reported that female rats fed a high-fat diet displayed higher levels of hepatic steatosis than males [39, 40]. Those differences could be due to the length of the experiment or to the age of the animals, as older animals might exhibit distinct metabolic adaptations and hormonal profiles that influence lipid metabolism differently compared to the 12-week-old rats used in the current study [41].

Male rats under long photoperiod exposure also presented the highest percentage of macrosteatosis. This can be associated with more advanced stages of hepatic steatosis and can compromise hepatocellular function by altering membrane integrity and mitochondrial activity, as macrovesicular steatosis is widely recognized as a hallmark of metabolic dysfunction [42, 43]. Studies have shown that macrovesicular steatosis is closely linked to hepatic insulin resistance, oxidative stress, and progression toward non-alcoholic steatohepatitis (NASH), all of which contribute to metabolic dysfunction [44, 45]. Consistent with the predominance of macrosteatosis observed in this group, one male rat under L18 photoperiod conditions presented lipogranuloma, which may reflect a more persistent hepatic injury. This is a sign of an active inflammatory and phagocytic response to excessive lipid accumulation and oxidative stress, in addition to its association with chronic injury and progression toward steatohepatitis in experimental NAFLD models [46, 47]. Moreover, almost all the rats in all the groups presented microgranuloma, which may reflect early or mild inflammatory responses to lipid accumulation, representing an initial protective mechanism against hepatocellular damage [48]. In addition, the higher proportion of microsteatosis in females compared to males, especially under short photoperiod conditions, may represent a milder or adaptive response to lipid overload. In this sense, microsteatosis usually reflects mild metabolic disturbances rather than structural damage, as it is often considered an earlier or potentially reversible form of lipid accumulation [42, 49].

The AST/ALT levels in the liver did not correlate with the degree of steatosis, suggesting that ALT and AST in this case are indicative of other forms of liver stress or injury, such as oxidative or mitochondrial dysfunction, inflammatory signaling, or early adaptive responses to metabolic overload [50, 51]. Indeed, while males exposed to L18 photoperiod exhibited the highest degree of hepatic steatosis, AST levels remained stable across all groups, and ALT was only slightly elevated in males under L6 photoperiod when compared to females. This discrepancy likely reflects that AST and ALT mainly indicate hepatocellular injury rather than simple fat accumulation. In contrast, females showed higher AST/ALT ratios than males despite lower fat content, which may result from sex-specific differences in hepatocyte metabolism and stress responses [52, 53]. Such dissociation between the degree of steatosis and transaminase levels has been previously reported in rats fed a high-fat diet [54].

Consistent with the higher degree of steatosis, males exposed to L18 photoperiod also exhibit the greatest lipid accumulation in the liver, consequential to a higher level of H-TC and H-TAG than females exposed to the same photoperiod conditions

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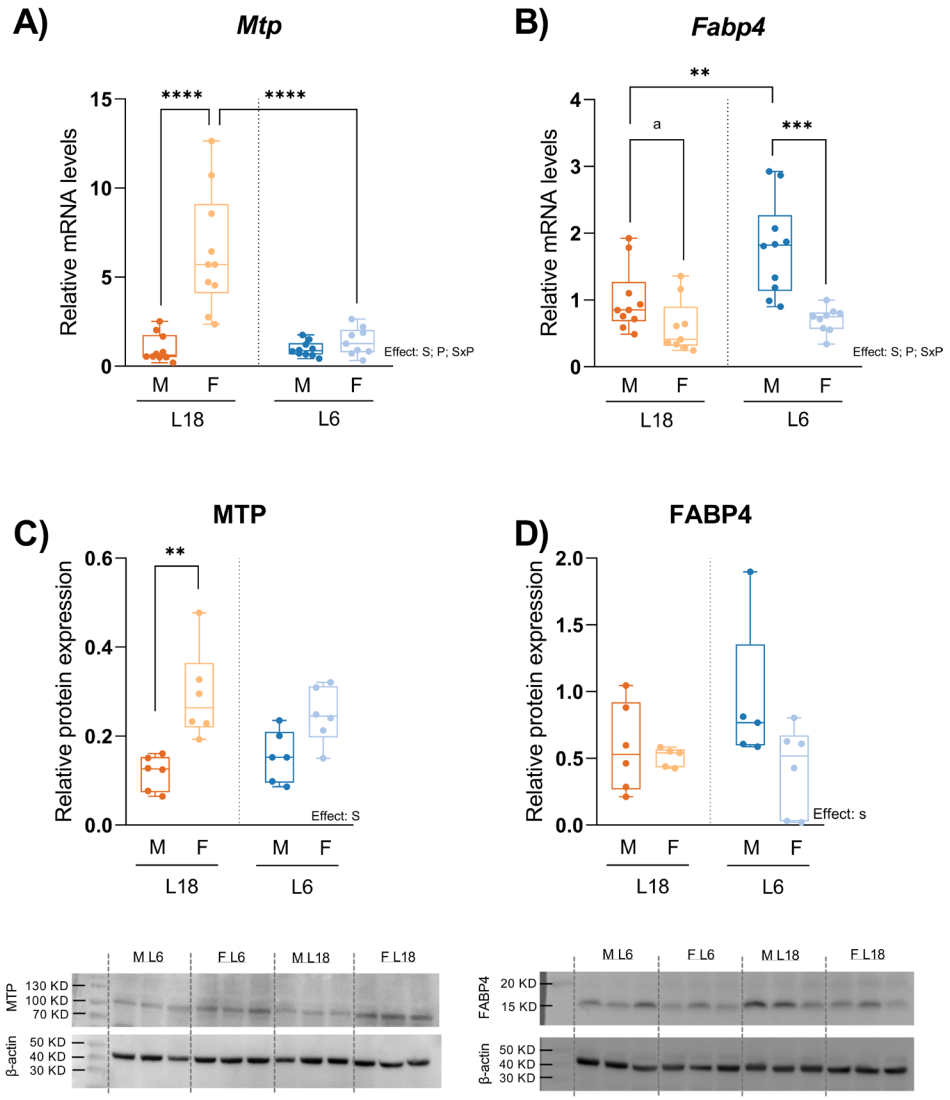

FIGURE 7 | Effect of sex and photoperiods on the relative gene and protein expression of lipid transport-related genes in the liver. Relative hepatic mRNA of *Mtp* (A) and *Fabp4* (B) and relative protein expression levels of MTP (C) and FABP4 (D) in male and female F344 rats fed with CAF diet for 11 weeks and exposed to L18 or L6 for the last 8 weeks. Data are expressed as minimum to maximum values, median and interquartile range ($n = 10$ for relative hepatic mRNA and $n = 6$ for relative protein expression levels). P, photoperiod effect; S, sex effect; S × P, interaction between sex and photoperiod assessed using two-way ANOVA for Fabp4, MTP and FABP4, and two-way non-parametric ANOVA with ART transformation for *Mtp*. *Indicates significant differences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$) and "a" indicates a trend ($p < 0.1$) between groups using two-way ANOVA followed by Tukey post hoc test for *Fabp4*, MTP and FABP4, and post hoc analysis with Tukey adjustment using emmeans package in R for *Mtp*. CAF, cafeteria; F, females; F344, Fischer 344; *Fabp4*, fatty acid binding protein 4; M, males; L6, short photoperiod of 6 h of light per day; L18, long photoperiod of 18 h of light per day; *Mtp*, microsomal triglyceride transfer protein.

and males under L6 photoperiod. In a recent study comparing male rats in different photoperiods, a trend toward higher H-TC levels was observed when rats were exposed to L18 conditions, which is in line with our data [55]. Moreover, CAF-fed rats showed increased H-TAG levels while maintaining similar levels of H-TC and H-PL when compared to standard-chow-fed rats, regardless of the photoperiod to which they were exposed [36, 56]. Moreover, it has been shown that female rats fed a high-fat diet under standard light–dark conditions present higher levels of H-TC than males [24]. This discrepancy with our data may be due to the age of the rats, which were 2 weeks younger, and to the duration of exposure to the CAF diet, which was 9 weeks, as this diet induces metabolic alterations in a time-dependent manner.

Female rats exhibited a healthier lipid profile compared to males, which is consistent with prior studies highlighting sexual dimorphism in metabolic regulation [57, 58]. This healthier lipid profile could be attributed to the higher levels of prolactin and corticosterone reported in females, which have a protective role, whereas males are described to present a higher concentration of insulin and triiodothyronine (T3), which contribute to lipid accumulation [58, 59]. Moreover, in a previous work with the animals used in this study, females presented a higher overall adiposity index than males [60]. However, this did not translate into worse metabolic outcomes, likely due to the protective effects of estrogens, which may mitigate the metabolic risks commonly associated with increased fat accumulation, such as insulin resistance and fatty liver disease

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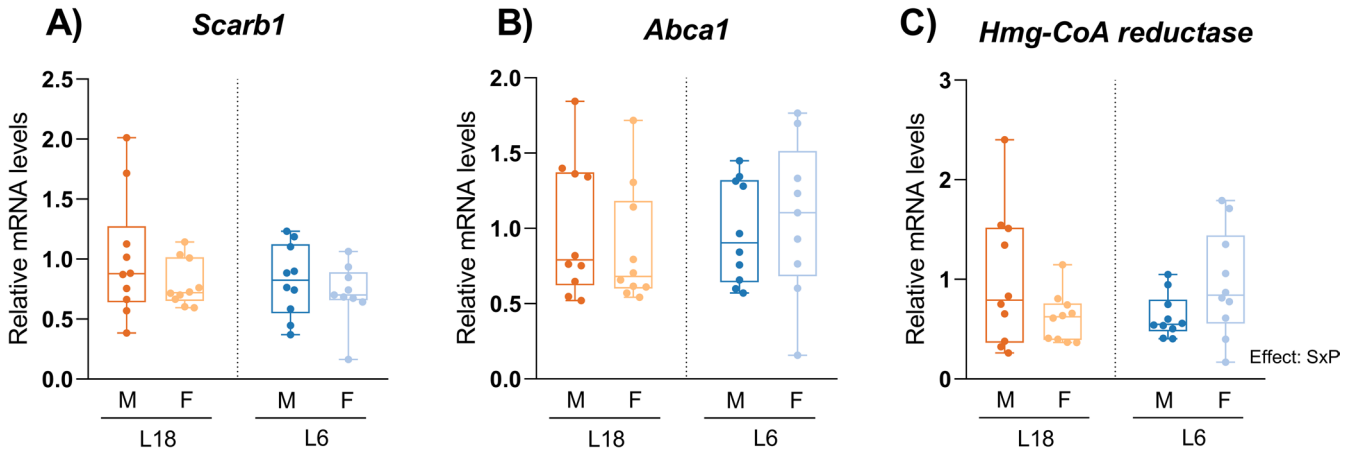

FIGURE 8 | Effect of sex and photoperiods on the relative gene expression of genes involved in hepatic cholesterol metabolism. *Scarb1* (A), *Abca1* (B), and *Hmg-CoA reductase* (C) relative hepatic mRNA levels in male and female F344 rats fed with CAF diet for 11 weeks and exposed to L18 or L6 for the last 8 weeks. Data are expressed as minimum to maximum values, median and interquartile range ($n = 10$). S×P, interaction between sex and photoperiod assessed using two-way ANOVA for *Abca1* and *Hmg-CoA reductase*, and two-way non-parametric ANOVA with ART transformation for *Scarb1*. *Abca1*, ATP-binding cassette transporter 1; CAF, cafeteria; F, females; F344, Fischer 344; *Hmg-CoA reductase*, 3-hydroxy-3-methylglutaryl-CoA reductase; L6, short photoperiod of 6 h of light per day; L18, long photoperiod of 18 h of light per day; M, males; *Scarb1*, scavenger receptor class B member 1.

[61, 62]. Furthermore, there were no differences in visceral fat or subcutaneous fat between males and females [60] even though it is reported that males tend to accumulate more visceral fat, associated with higher risks of metabolic diseases such as insulin resistance and fatty liver disease, than females, who tend to have more subcutaneous fat [57, 58]. In this work, the gene expression analysis provides key insights into the molecular mechanisms underlying sexual differences. The most significant gene expression difference was observed for *Mtp*, which exhibited a much higher expression in females under L18 conditions when compared to the other groups. MTP is a key regulator of hepatic lipid homeostasis, facilitating lipid export by promoting VLDL assembly and secretion, thereby preventing H-TAG accumulation [63–65]. The marked upregulation of *Mtp* observed in this group at the gene expression level was confirmed at the protein level, supporting a coordinated transcriptional and translational regulation of this lipid export pathway. The marked upregulation of MTP in females under L18 photoperiod may be the key point in their hepatic lipid homeostasis, evidenced by several metabolic advantages. On one hand, H-TAG levels are reduced in female rats at L18 as a potential effect of MTP upregulation. On the other hand, the elevated bile acid levels in female L18 rats suggest that increased bile acid synthesis promotes biliary excretion of cholesterol, thus minimizing intrahepatic lipid retention and the severity of steatosis [66]. However, females under L18 presented slightly higher hepatic steatosis compared to females under L6 photoperiod. This contrast could be partially explained by the expression levels of *Srebp-1c*, a key transcription factor regulating de novo lipogenesis. Notably, *Srebp-1c* was downregulated in females exposed to L6 photoperiod, suggesting reduced lipogenic activity and contributing to the lower degree of hepatic steatosis observed in this group. This finding is consistent with research showing that estrogens can modulate hepatic lipid metabolism and bile acid synthesis [67]. Estrogens have been shown to exert hepatoprotective effects by enhancing bile acid synthesis and lipid export via upregulation of *Mtp*, as well as by their antioxidative and anti-inflammatory properties [67, 68]. Furthermore, these results are in concordance with previous studies, in which estrogen levels are affected by photoperiod conditions [69]. Estrogen can also explain the lower levels of bilirubin in females compared to males exposed to L6 conditions, due to their hepatoprotective role [70]. This effect might involve photoperiod-induced changes in bilirubin metabolism enzymes [71, 72]. On the other hand, in females, although melatonin levels are elevated under L6, the protective effect of melatonin on hepatic lipid profile is not evidenced, likely due to the additional modulatory role of estrogens [23]. Notably, our group previously reported that female rats inherently exhibit a more favorable hepatic lipid profile compared to males, which may contribute to the smaller magnitude of photoperiod-induced changes observed in females [60].

Males in L6 showed reduced total hepatic lipids, hepatic triglycerides, and a tendency to reduce hepatic cholesterol levels compared to L18-exposed males, coinciding with a lower degree of hepatic steatosis. In this sense, males in L6, showed an upregulation of mRNA levels of *Fabp4* compared to female rats, suggesting a sex-specific response to diet-induced elevated plasmatic NEFA levels. Given the intracellular fatty acid transport role of *Fabp4*, this upregulation may reflect an increased hepatic capacity for NEFA uptake and intracellular trafficking, rather than their storage or secretion via [11] However, the upregulation of *Fabp4* mRNA levels in males under L6 conditions, was not supported by significant changes in protein expression. In addition, the expression of genes involved in β-oxidation, such as *Cpt-1α* and *Ppar-α*, was also downregulated in males in L6, indicating that fatty acids were not primarily oxidized in the liver, but may be rather redirected for peripheral use. Males exposed to L6 are indeed characterized by prolonged and elevated nocturnal melatonin levels and these findings align with recent rodent studies demonstrating that melatonin administration attenuates hepatic steatosis by modulating β-oxidation pathways and promoting fatty acid redistribution away from

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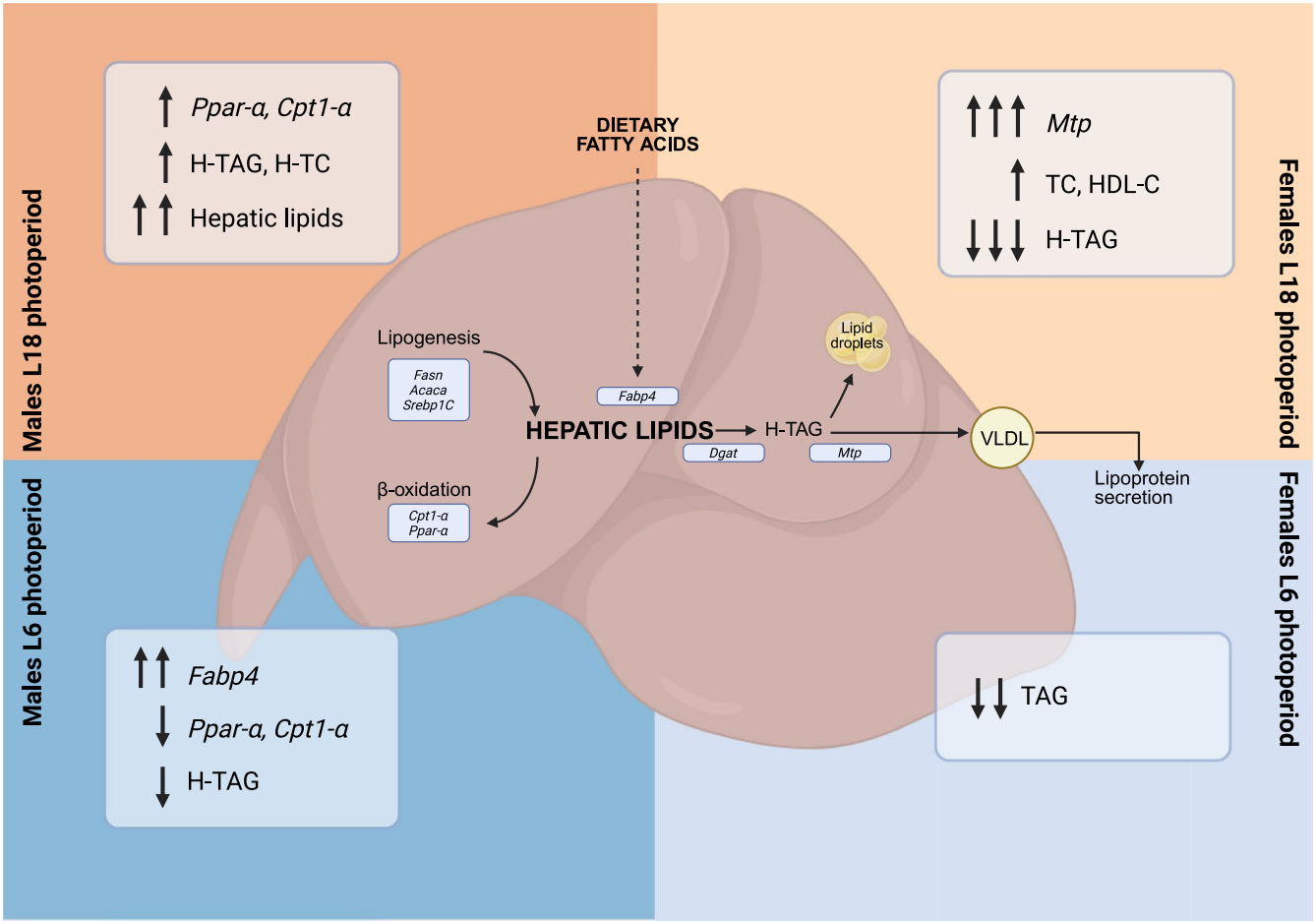

FIGURE 9 | Summary of the main metabolic pathways implicated in the management of the hepatic lipids in male and female F344 rats fed with CAF diet for 11 weeks and exposed to L18 or L6 for the last 8 weeks. *Acaca*, acetyl-CoA carboxylase alpha; CAF, cafeteria; *Cpt-1α*, carnitine palmitoyltransferase I alpha; *Dgat*, diacylglycerol *O*-acyltransferase 1; F344, Fischer 344; *Fabp4*, fatty acid binding protein 4; *Fasn*, fatty acid synthase; HDL-C, high-density lipoprotein cholesterol; H-TAG, hepatic triglycerides; H-TC, hepatic total cholesterol; L18, long photoperiod of 18 h of light per day; L6, short photoperiod of 6 h of light per day; *Mtp*, microsomal triglyceride transfer protein; *Ppar-α*, peroxisome proliferator-activated receptor alpha; TAG, triglycerides; TC, total cholesterol; VLDL, very-low-density lipoprotein.

the liver [73, 74]. Furthermore, males under L6 also exhibited a downregulation of the lipogenesis-related gene *Dgat*, when compared to females. This coordinated suppression of both lipid synthesis and oxidation likely reflects an adaptive hepatic response to the increased energy demand associated with higher hours of darkness and activity under L6 photoperiod conditions. By facilitating fatty acid redistribution rather than promoting internal storage or catabolism, the liver contributes to the maintenance of hepatic lipid homeostasis [11, 75]. Figure 9 summarizes the main metabolic pathways implicated in the hepatic lipid management in male and female rats exposed to short and long photoperiods.

5 | Conclusion

This study highlights a sex-specific adaptation to seasonal photoperiods in hepatic lipid handling in rats under obesogenic conditions. Specifically, females generally exhibited a healthier lipid profile and under L18 photoperiod conditions a lower hepatic lipid accumulation compared to males. These differences were reflected in the upregulation of the *Mtp* gene in females exposed to L18, promoting lipid export and reducing H-TAG levels, whereas increased hepatic *Fabp4* expression in males exposed to L6 photoperiod suggests fatty acid mobilization toward peripheral tissues rather than its hepatic accumulation, resulting in a lower degree of hepatic steatosis.

These findings underscore the importance of considering both sex and seasonal light variations when studying metabolic disorders and their potential treatments, contributing to more personalized approaches in metabolic health management and offering valuable insights for future chronobiological studies in human populations.

Author Contributions

Saioa Gómez-Roncal: investigation, formal analysis, validation, writing – original draft preparation, writing – review and editing. **Fabiola C. García-Reyes:** investigation. **Jorge R. Soliz-Rueda:** investigation. **Enrique Calvo:** funding acquisition. **Manuel Suárez:** funding acquisition. **Begoña Muguerza:** funding acquisition. **Anna Arola-Arnal:** methodology, conceptualization, project administration, supervision, funding acquisition, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data available on request from the authors.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Primers sequences used for gene analysis in liver.

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