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Sweet cherries modulate thyroid hormones and hepatic glucose metabolism in F344 rats in a photoperiod-dependent manner

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ABSTRACT

Sweet cherry intake affects oxidative stress and hepatic lipid metabolism, depending on composition, mainly (poly)phenol profile and daylight length. Thyroid hormones (TH) respond to daylight changes and regulate the hepatic glucose metabolism. This study investigated whether cherries with different phenolic hallmarks exhibit seasonal responses to TH and hepatic glucose metabolism. Fischer 344 rats fed a standard diet were chronically exposed to L6 (6 h light), L12 (12 h light), and L18 (18 h light) photoperiods and were supplemented with two sweet cherries (Ch1 and Ch2). Serum TH levels and glucose metabolism-related metabolites were affected by both the cherry composition and photoperiod. Thus, Ch1 and Ch2 reduced serum T3 levels in L6, whereas Ch2 increased it in L18, nullifying the photoperiod effect. Moreover, although photoperiod was the main factor affecting the hepatic expression of gluconeogenesis-related genes, Ch1 exerted effects mainly on L6 photoperiod and Ch2 on L18, tending to cancel out the photoperiod effects.

1. Introduction

Most vertebrates adapt their physiology to external conditions as a survival response to seasonal changes (Ebling & Barrett, 2008). Despite their short lifespan, seasonal variations in reproduction, body weight (BW), and food intake have been shown to occur even in small animals (Dardente, 2015). In addition, different tissues have peripheral patterns that are sensitive to the photoperiod. Specifically, chronic exposure to a short photoperiod (6 h light/18 h dark) resulted in lower serum insulin levels and higher serum glucose levels in rats fed a standard diet than those exposed to a long photoperiod exposure (18 h light/6 h dark), together with the downregulation of genes involved in glucose metabolism and insulin signalling (Mariné-Casadó et al., 2019).

Day length is one of the main seasonal cues (Thomas, 2016). The change in photoperiod affects nocturnal melatonin production in the pineal gland, which in turn stimulates the production of thyroid-stimulating hormone (TSH) in the hypophysis (*pars tuberalis)* and controls the availability of local thyroid hormone (TH) in hypothalamic tanycytes by acting on deiodinase (DIO) enzymes (Ross, Helfer, Russell, Darras, & Morgan, 2011). This photic information is translated as a loss of BW and a decrease in food intake during a short photoperiod in Fisher 344 (F344) rats (Tavolaro et al., 2015). The active form of TH, triiodothyronine (T3), plays a variety of physiological roles associated with metabolism, thermogenesis, heart rate (Mullur et al., 2014), and glucose metabolism in the liver (Sinha et al., 2014).

Eating cherries, seasonal fruits consumed between spring and summer, have been linked to health-promoting effects, such as protection against high-fat-induced steatosis in the liver, and antioxidant and anti-inflammatory properties (Faienza et al., 2020; Ferretti, Bacchetti, Belleggia, & Neri, 2010; Kent et al., 2016). Moreover, their beneficial

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effects were photoperiod-dependent (Mariné-Casadó et al., 2019). Regarding to the molecules responsible of their beneficial effects, cherries are a source of vitamins, minerals, tryptophan and, particularly, phenolic compounds (Cory, Passarelli, Szeto, Tamez, & Mattei, 2018; Garrido et al., 2013). The phenolic profile of cherries has been extensively studied, containing high levels of hydroxycinnamic acids, anthocyanins, flavan-3-ols, and flavonols, but their profiles varies according to environmental conditions (temperature, humidity, light, soil, etc.), maturity, processing, and storage (Iglesias-Carres et al., 2019; Martini et al., 2017). As a matter of fact, cherries from different geographical origins showed differences in the abundance of total phenolic compounds, anthocyanins, and flavanols (Cruz-Carrión et al., 2020). In addition, the administration of these cherries with different phenolic hallmarks to F344 rats subjected to different photoperiods exerted dissimilar effects on oxidative stress (Cruz-Carrión et al., 2020), hepatic lipid metabolism, which is in turn regulated by TH (Ruiz de Azua, Cruz-Carrión, Muguerza, Arola-Arnal, & Suarez, 2021), and hypothalamic genes associated with the photoperiodic response that affects body weight and food intake and genes linked to hippocampal neuroprotection (Manocchio, Bravo, Helfer, & Muguerza, 2024).

Considering all the evidence, the purpose of the present study was to study the effect of the consumption of cherries with different phenolic signatures on both TH levels and hepatic glucose metabolism in F344 rats exposed to different photoperiods.

2. Material and methods

2.1. Chemical and reagents

Liquid chromatography coupled to triple quadrupole mass spectrometry (LC-QqQ) analysis standards; L-thyroxine-d4, L-thyroxine (T4), 3,3′,5-triiodo-L-thyronine (T3), and 3,3′,5′-triiodo-L-thyronine-13C6 solution were provided by Sigma-Aldrich (St. Louis, MO, USA). Water (Sharlab, Barcelona, Spain), acetic acid (Sigma-Aldrich), methanol (MERK, Madrid, Spain), and ammonium acetate (Sigma-Aldrich) were HPLC grade. TRIzol reagent was purchased in Thermo Fisher Scientific (Illkirch Graffenstaden, France) and Universal SYBR Green Supermix in Biorad (Madrid, Spain). All other reagents were of analytical grade.

2.2. Sweet cherries

Brooks sweet cherries (*Prunus avium* L.) from different geographical locations, Spain (Ch1) and Chile (Ch2), were used in this experiment to obtain cherries with different phenolic hallmarks. The frozen cherries were lyophilized after being ground and kept at −20 °C until use. Basic composition and phenolic content were previously quantified by Cruz-Carrión et al. (2020). In addition, phenolic compounds were extracted using the same methodology described by Iglesias-Carres et al. (2019), (72 % methanol containing 1 % formic acid preheated at 55 °C), which were further analysed using HPLC-ESI-MS/MS, using the same equipment, methodology, and calibration curves described by Iglesias-Carres et al. (2018, 2019). Results revealed that anthocyanins were the dominant (poly)phenol family in these fruits, comprising 52 % and 75 % of the total (poly)phenol content in Ch2 and Ch1, respectively. Flavanols, flavonols, and phenolic acids were also present. Considering the major phenolic compounds found, Ch1 had higher levels of cyanidin-3-*O*-rutinoside (3.42 times), catechin (3.20 times), and 3-*O*-caffeoylquinic acid (1.33 times) while Ch2 had higher levels of quercetin-3-*O*-rutinoside (1.18 times) and 4-hydroxybenzoic acid (1.04 times) (data unpublished). Cherry characterization was carried out at the same time as animal study.

2.3. Experimental design

F344 male rats (between 7–8 weeks of age) were supplied by Charles River Laboratories (Barcelona, Spain). Animals were housed at 22 °C, two rats per cage, and divided into three photoperiods: L18 (long; 18 h light/6h dark), L12 (intermediate; 12 h light/12 h dark), and L6 (short; 6 h light/18 h dark), which simulate the seasons. The rats had unlimited access to tap water and a standard chow diet (AO4, Panlab, Barcelona, Spain) during all experiment. During the four-weeks period of adjusting to a light–dark cycle, rats learned to lick water from a syringe (way to administer the treatments). Then, rats were subdivided into three groups (n = 8 per group) and supplemented daily for 7 weeks with 100 mg of two cherries (dry weight, dw)/kg BW (Ch1 group and Ch2 group), suspended in tap water. Doses were prepared daily by weighing the freeze-dried cherry powders and administered to animals through a syringe by voluntary licking (0.3–0.4 mL) and ensuring that the animals consumed the correct dose. A dose of 100 mg/kg was selected because previous studies have shown that this dose can produce different metabolic effects in F344 (Cruz-Carrión, de Azua, Mulero, Arola-Arnal, & Suárez, 2020; Mariné-Casadó et al., 2019; Ruiz de Azua, Cruz-Carrión, Muguerza, Arola-Arnal, & Suarez, 2021). Considering the moisture content of the cherries (80–82 %) and the formula of Reagan-Shaw et al. (2008) to translate dose from animals to humans (weight of 75 kg and height of 175 cm), the cherry dose administered to rats would be equivalent to a dose of 7.6 g of fresh cherries per day in humans. Moreover, control group (C group) was administered 42.4 mg/kg BW of a solution containing glucose and fructose (1:1, w/w; vehicle), to simulate the sugar content contained in cherries. Because the vehicle provides 0.11 % of the daily energy, its effect is not considered relevant because of its low energy value. During the experiment, rats were weighed weekly until the end of the experiment. After completing the experiment, rats were deprived of food for 1 h after receiving the treatments and were sacrificed 1 h post-treatment by decapitation. The serum was acquired by centrifuging non-heparinized blood at 2,000 × g for 15 min at 4 °C. The liver was immersed in liquid nitrogen immediately after collection and then stored at − 80 °C. The experimental design for this study is represented in Fig. 1.

The animal protocols used in this research were in compliance with the European Communities Council Directive (86/609/EEC) and were approved by the Animal Ethics Review Committee for Animal Experimentation of the Universitat Rovira i Virgili and the Generalitat de Catalunya. The permission numbers for this study were 9495 and the FUE-2017–00499873, respectively.

2.4. Determination of serum thyroid hormones

Serum content of TH (T3 and T4) were assessed using LC-QqQ according to the method and equipment reported by Soliz-Rueda et al., (2022). Preparation of samples was as follows: methanol (250 μL) containing 2 ng/mL of the internal standards (L-thyroxine-d4 and 3,3′,5′-Triiodo-L-thyronine-13C6 for T4 and T3, respectively) was added to serum (50 μL). After centrifugation at 27,670 x g for 5 min at 4 °C, 0.1 % formic acid (700 μL) was added to the supernatant and the solution was introduced into a conditioned Oasis HLB 96-wells plate (Waters Milford, MA, USA). The serum compounds were eluted with methanol (500 μL), which was evaporated using a SpeedVac at 45 °C. Then, remained compounds were dissolved with 50 μL of Milli-Q water: methanol (60:40, v/v). Compounds were separated using a C18 analytical column (Zorbax Eclipse, 150 x 2.1 mm, Agilent Technologies) included in a spectrometric Ultra-High-Performance Liquid Chromatography (1290 Infinity II Series; Agilent Technologies) coupled to a QqQ/MS (6490 Series; Agilent Technologies). Volume of injection, column temperature, and flow rate were set to 5 μL, 45 °C and 3 mL/min, respectively. Calibration curves were generated for T4 and T3 in the range of 1–100 ng/mL and 0.05–5 ng/mL, respectively.

2.5. Gene expression analysis

RNA extraction from liver tissue was performed using TRIzol reagent, according to the manufacturer's instructions. cDNA was

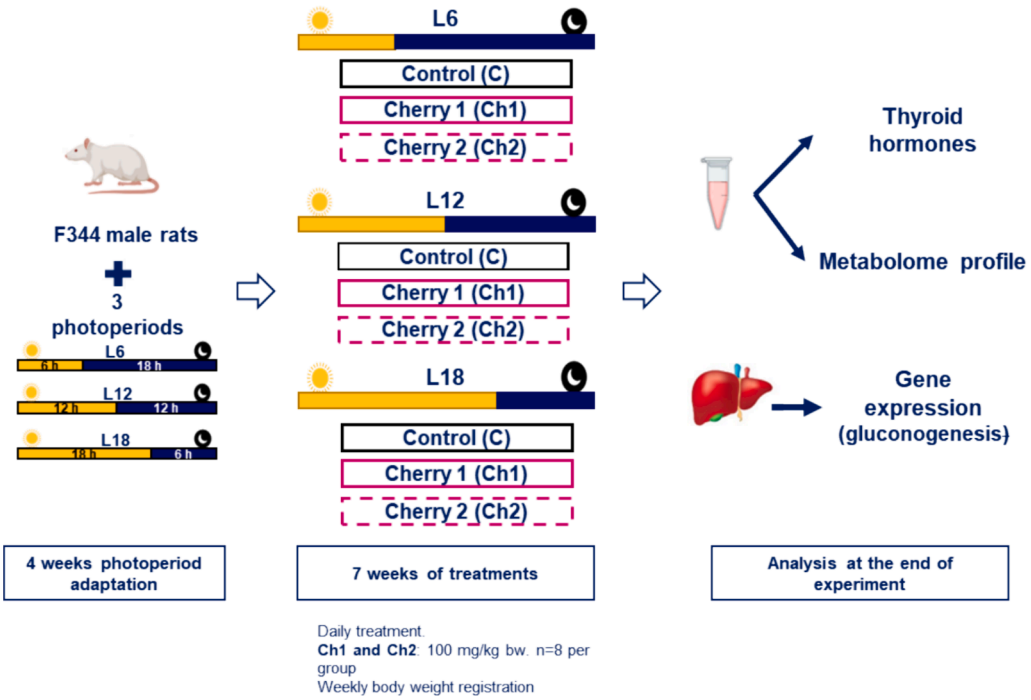

Fig. 1. Experimental design (created with BioRender.com).

synthesized through reverse transcription using a high-capacity cDNA reverse transcription kit from Thermo Fisher Scientific. Amplification of specific cDNA fragments was performed using RT-qPCR) and iTaq Universal SYBR Green Supermix. Specifically, glucose-6-phosphatase catalytic subunit *(G6pc)*, phosphoenolpyruvate carboxykinase 1 *(Pck1)*, iodothyronine deiodinase 1 *(Dio1),* glucokinase *(Gk),* glucose transporter 2 *(Glut2),* and nicotinamide phosphoribosyltransferase *(Nampt)* genes. Relative expression levels were determined by expressing them as a percentage of the L12 Control group, with peptidylprolyl isomerase A (*Ppia*) serving as a reference gene. The nucleotide sequences of primers used were provided by Bioprimers (Ulm, Germany) and are listed in Table S1.

2.6. Serum extraction and ^{1}H NMR spectrometry

Proton Nuclear Magnetic Resonance (^{1}H NMR) was used to analysed serum metabolites as previously described by Palacios-Jordan et al., (2020) and using the same equipment. Hydrophilic metabolites were obtained using a twice-steps process, in which firstly, serum was mix with methanol and Milli-Q water to obtain a monophasic solution, and secondly, this solution was mix with chloroform and Milli-Q water. After centrifugation, the upper aqueous phase was dried using the freeze-drying method and stored at − 80 °C. Analysis of ^{1}H NMR spectra was carried out using the Chenomx NMR suite 8.4 software (Chenomx Inc., Edmonton, AN, Canada) and the AMIX 3.9 software package. Metabolite identification was performed using pure compound references obtained from the metabolic profiling AMIX spectra database (Bruker) and other databases.

2.7. Statistical analysis

Data were presented as the mean ± standard deviation. SPSS Statistics 22 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis and outliers identification. Differences between groups were identified using one-way ANOVA ($p < 0.05$, Tukey's post hoc test;) and two-way ANOVA ($p < 0.05$, Tukey's post-hoc test): photoperiod (*P*), fruit (*F*), or interaction of both factors (*PxF*).

3. Results

3.1. Photoperiod intake of sweet cherries and their effects on body weight

The evolution of BW and BW gain of animals subjected to the different photoperiods and supplemented C, Ch1, and Ch2 during the study are shown in Figures 2 and S1, respectively. As expected, the BW increased in all groups during the 11 weeks of study (Fig. 2). At the end of the experiment, the L6-C group showed a tendency for decreased BW compared to the L12-C group (Fig. 2A). Ch1 significantly lowered BW at L6 compared to that at L12 (Fig. 2B). No differences were observed between photoperiods neither when rats consume Ch2 (Fig. 2C) or when the three groups were compared for each photoperiod. However, differences found between groups in BW were not observed in BW gain (Figure S1).

3.2. Photoperiod intake of sweet cherries and their effects on thyroid hormones

Interactions between photoperiod and cherry effect were observed in serum T3 and T4 levels ($p = 0.002$ and $p = 0.03$, respectively, two-way ANOVA: *PxF*). T3 content significantly increased in L6-C group (119.0 ng/dL) respect to L12-C (100.2 ng/dL) and L18-C (87.7 ng/dL) groups (Fig. 3A). Moreover, cherry effect consumption was observed in both L6-Ch1 and L6-Ch2 by decreasing in a different manner T3 levels. No differences were observed when the cherries were consumed in L12; however, Ch2 consumption increased T3 levels in L18 compared to the L18-C and L18-Ch1 groups, nullifying the photoperiod effect (Fig. 3A). Regarding T4 levels, a very similar pattern was observed for the active thyroid hormone (T3). A photoperiod effect was observed when L6-C (5.1 μg/dL) and L18-C (4.0 μg/dL) T3 levels were compared. In addition, L6-Ch1 group had lower serum T4 levels than that in the L6-C group (Fig. 3B). Similar to T3, the L18-Ch2 group showed higher T4 levels than the L18-C group. To study the hepatic conversion of T4 to T3, hepatic *Dio1* mRNA expression was determined. Photoperiod effect was also observed in hepatic *Dio1* mRNA expression ($p < 0.05$, two-way ANOVA: *P*) as L6-C animals showed a significantly upregulated hepatic *Dio1* expression in comparison with L12-C (Fig. 3C), and cherry effect

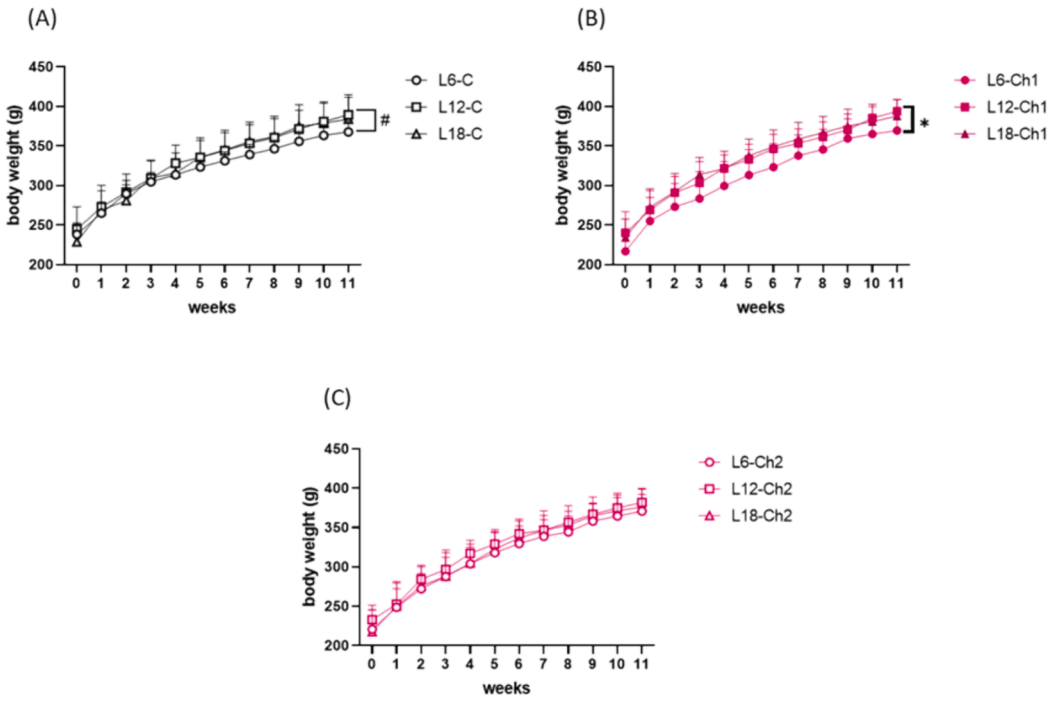

Fig. 2. Body weights of Fisher 344 rats exposed to L6 (6 h light/ 18 h dark), L12 (12 h light/ 12 h dark), or L18 (18 h light/ 6 h dark) photoperiods for 11 weeks and administered vehicle (C, figure A), cherry 1 (Ch1, 100 mg/kg body weight, figure B) or cherry 2 (Ch2, 100 mg/kg body weight, figure C) for the last 7 weeks. * and # indicate significant differences ($p < 0.05$) and tendency ($0.05 < p < 0.1$) between the L6 group and the L12 and L18 groups at the 11th week (one-way ANOVA, post-hoc Tukey's test).

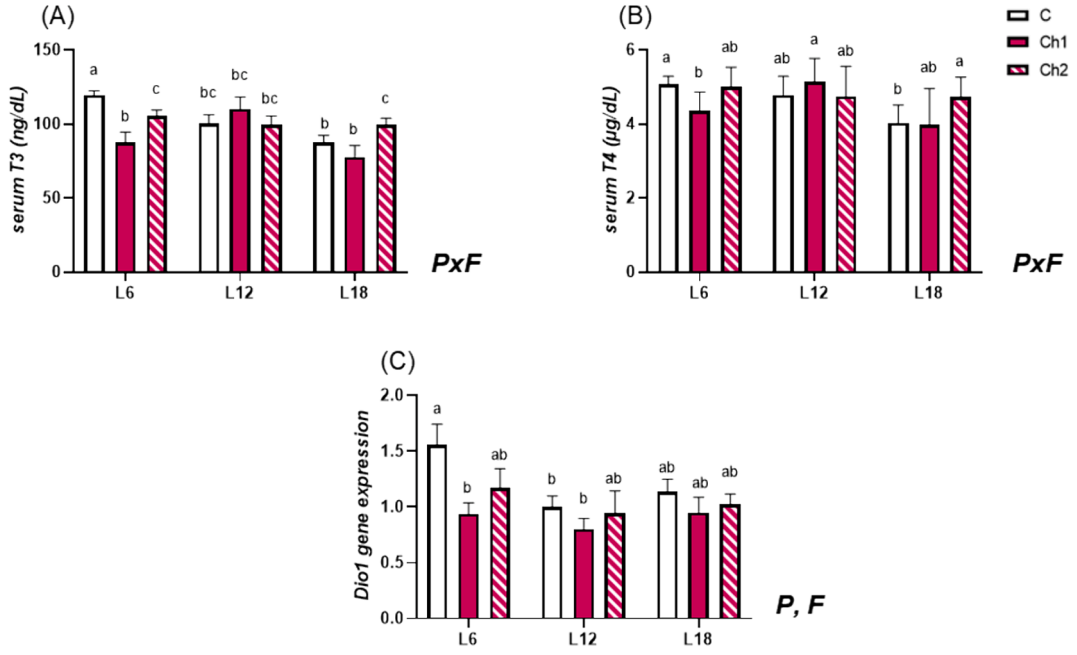

Fig. 3. Effect of photoperiod and cherry consumption on thyroid hormone levels. Serum thyroid hormone levels triiodothyronine (T3, A) and thyroxine (T4, B) and hepatic expression levels of *Dio1* of Fisher 344 rats exposed to L6 (6 h of light/ 18 h of dark), L12 (12 h of light/ 12 h of dark) or L18 (18 h of light/ 6 h of dark) photoperiods for 11 weeks and administered vehicle (C), cherry 1 (Ch1, 100 mg/kg body weight) or cherry 2 (Ch2, 100 mg/kg body weight) during the last 7 weeks. Values are expressed as the mean ± standard deviation (n = 8 per group). *P, F,* or *PxF* indicate significant differences produced by photoperiod, fruit, or interaction between photoperiod and fruit, respectively ($p < 0.05$, two-way ANOVA,). Different letters indicate significant differences ($p < 0.05$, one-way ANOVA, post-hoc Tukey's test).

was observed when Ch1 was consumed in L6, counteracting the effect of the photoperiod.

3.3. Photoperiod intake of sweet cherries and their effects on serum metabolic profile

Serum metabolomic profiles were determined using high-resolution

^{1}H NMR spectroscopy (Table S2), and metabolites related to gluconeogenesis were selected and plotted in heatmaps (Fig. 4). In the L6 photoperiod, Ch2 consumption increased the level of serum metabolites compared with the Control and Ch1 groups. No remarkable shifts were observed in L12 photoperiod; however, some metabolite levels, such as valine and asparagine were differentially altered by cherry consumption. In L18, both Ch1 and Ch2 showed notably different patterns compared to those in L18-C. Statistical analyses (one- and two-way ANOVA) are presented in Table S2.

3.4. Photoperiod intake of sweet cherries and their effects on hepatic genes

A subset of genes related to gluconeogenesis (*Glut2, Gk, Pck1*, *G6pc*, and *Nampt*) was analysed in the liver (Fig. 5). Hepatic *Glut2* expression did not exhibit a photoperiod expression pattern (Fig. 5A). Regarding cherry consumption, changes in *Glut2* expression were observed only in L6, where the L6-Ch2 group showed significantly higher expression than the L6-Ch1 and L6-C groups. In addition, gene expression analysis revealed that *Gk* is part of a subset of genes affected by photoperiod exposure ($p < 0.01$, two-way ANOVA,). L18-C showed higher *Gk* gene expression levels than both the L12-C and L6-C groups (Fig. 5B). The fruit consumption effect was observed on this hepatic gene only in L6, where Ch1 significantly downregulated, its expression compared with the L6-C group. As depicted in Fig. 5C and D, *G6Pc* (two-way ANOVA: P, $p < 0.001$; F, $p < 0.05$) and *Pck1* (two-way ANOVA: *P*, $p = 0.01$), which encode crucial gluconeogenic enzymes, were significantly downregulated in the L18-C when compared to the L6-C and L12-C groups. Regarding the cherry consumption effect, cherries upregulated the expression of both genes, counteracting the photoperiod effect on *G6Pc* (Fig. 5C and D). Specifically, Ch2 upregulated *Pck1* in L18-Ch2 animals in comparison with the L18-C group and upregulated *G6Pc* expression in Ch2-L6 and Ch2-L18, whereas Ch1 only upregulated *G6Pc* expression in the L6 photoperiod. Differences between the types of cherries were only observed at L18 for *Pck1* (Fig. 5D).

Finally, *Nampt* mRNA expression (Fig. 5E) was upregulated in the L18-C group compared to the L6-C and L12-C groups, highlighting the photoperiod effect (two-way ANOVA: *P*, $p = 0.007$). The cherries effect was observed in the L6 and L18 groups, as *Nampt* expression was downregulated in the L6-Ch1 group and in L18-Ch2 compared with L6-C and L18-C, respectively. L18-Ch2 animals showed levels of *Nampt* expression similar to those observed in the L12-C group. The differential effect of cherries on the expression of this gene was observed in L18 (Ch1 group vs. Ch2 group).

4. Discussion

Seasonal rhythms allow animals to adapt their physiology to the external environment, such as changes in light duration, temperature, and nutrient availability (Ebling, 2014). Previous studies in hamsters and F344 rats suggested that seasonal changes in the hypothalamus on local TH availability, among other factors, control energy balance (Ebling, 2020; Helfer & Tups, 2016). Nevertheless, the effects of the photoperiod on circulating TH levels and possible metabolic outcomes have not yet been described. In addition, recent research suggests that thyroid function can be regulated not only by iodine, but also by several food compounds including melatonin, vitamins, and phenolic compounds (Benvenga et al., 2019; Giuliani et al., 2014). Moreover, phenolic compounds can modulate both circadian and circannual rhythms (Arola-Arnal et al., 2019), a phenomenon explained by the xenohormesis theory (Baur & Sinclair, 2008). According to xenohormesis, plants produce phenolic compounds under stress conditions depending on the external environment, and consumers can sense these chemical cues, which in turn allows them to adapt to the environmental conditions (Howitz & Sinclair, 2008).

Crosstalk between nutrition and metabolism regulation in a

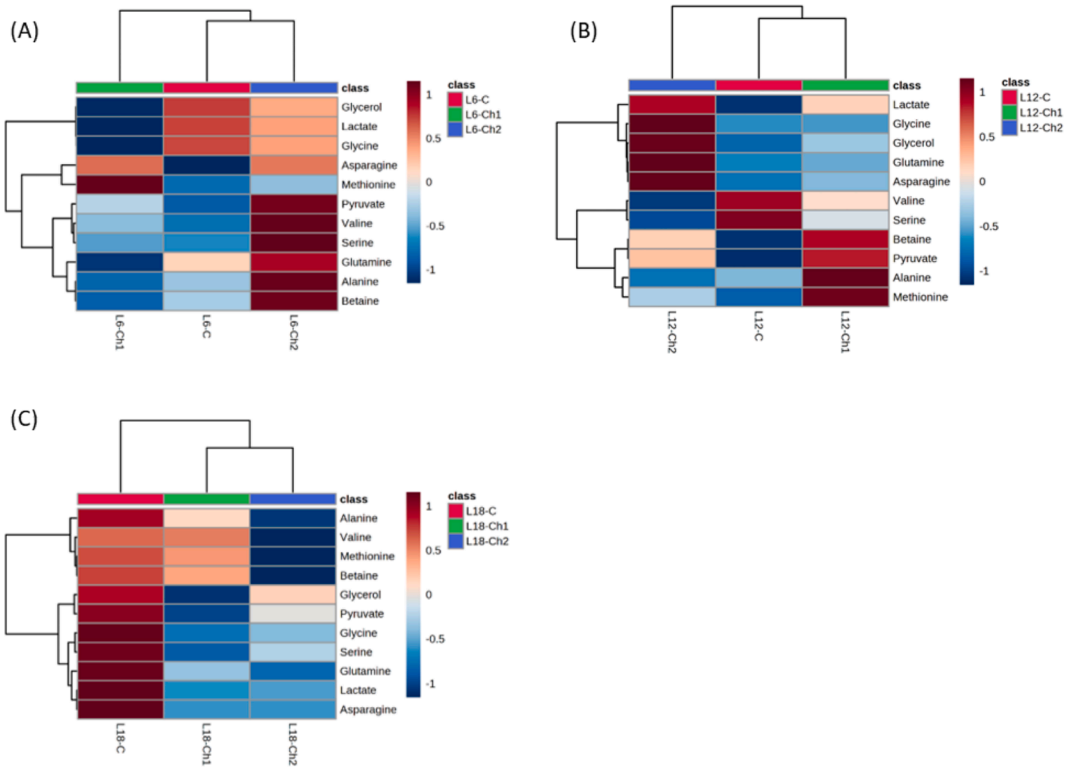

Fig. 4. Heatmap plot of hierarchical clustering analysis of the serum gluconeogenic-related metabolites of Fisher 344 rats exposed to L6 (6 h light/ 18 h dark, figure A), L12 (12 h light/ 12 h dark, figure B), or L18 (18 h light/ 6 h dark, figure C) photoperiods for 11 weeks and administered vehicle (C), cherry 1 (Ch1, 100 mg/kg body weight), or cherry 2 (Ch2, 100 mg/kg body weight) for the last 7 weeks (n = 8 per group).

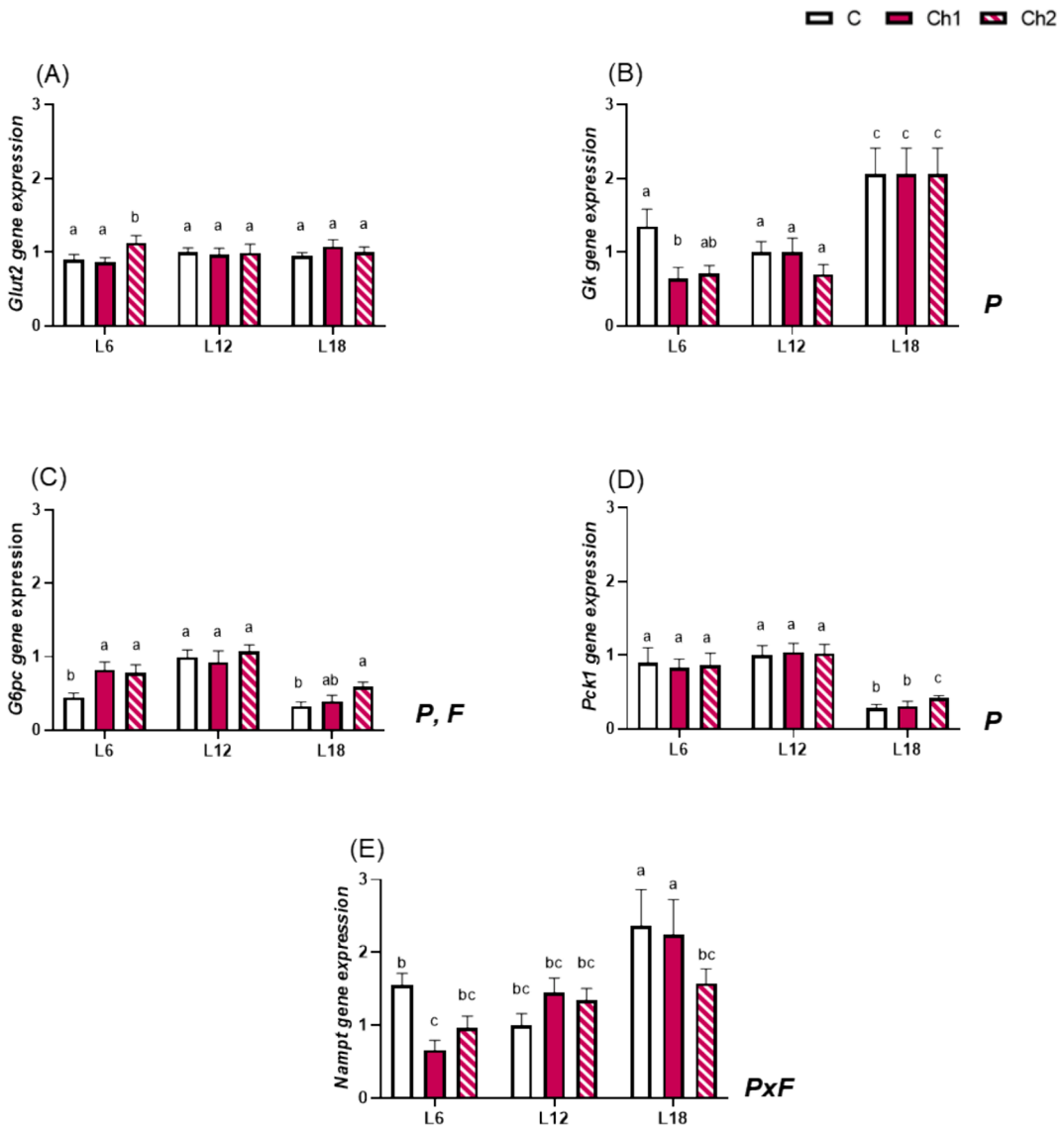

Fig. 5. Hepatic expression levels of *Glut2* (A), *Gk* (B), *G6pc* (C), *Pck1* (D), and *Nampt (E)* in Fisher 344 rats exposed to L6 (6 h light/ 18 h dark), L12 (12 h light/ 12 h dark) or L18 (18 h light/ 6 h dark) photoperiods for 11 weeks and administered vehicle (C), cherry 1 (Ch1, 100 mg/kg body weight), or cherry 2 (Ch2, 100 mg/kg body weight) for the last 7 weeks. *P, F* or *PxF* indicates significant differences produced by photoperiod, fruit or interaction between photoperiod and fruit, respectively ($p < 0.05$, two-way ANOVA). Different letters indicate significant differences ($p < 0.05$, one-way ANOVA, post-hoc Tukey's test). Abbreviations: *Gk* (glucokinase); *Glut2* (glucose transporter 2); *G6pc* (glucose-6-phosphatase catalytic subunit); *Nampt* (nicotinamide phosphoribosyl transferase); *Pck1* (phosphoenolpyruvate carboxykinase 1).

photoperiod-dependent manner remains an outstanding question. In recent years, our group observed that the administration of seasonal fruits rich in phenolic compounds, such as sweet cherries, to healthy animals adapted to different photoperiods produced different metabolic responses depending on the photoperiod of exposure (Ruiz de Azua, Cruz-Carrión, Muguerza, Arola-Arnal, & Suarez, 2021; Mariné-Casadó et al., 2019), and sweet cherries from different geographical origins exerted effects on hippocampal neuroprotective-related genes (Manocchio, Bravo, Helfer, & Muguerza, 2024) and liver lipid metabolism, which depended on the photoperiod and geographical origin (Ruiz de Azua, Cruz-Carrión, Muguerza, Arola-Arnal, & Suarez, 2021). Furthermore, the phenolic signatures of these cherries from different geographical origins may explain the differences in the effects exerted (Cruz-Carrión et al., 2020). Considering that TH have been described as a seasonal rhythmic generator (Dardente et al., 2014) and play a key role in linking the neuroendocrine response to peripheral organs by regulating lipid and glucose metabolism (Sinha et al., 2014), and sweet cherries have shown photoperiod-dependent effects on hepatic lipid metabolism (Ruiz de Azua, Cruz-Carrión, Muguerza, Arola-Arnal, & Suarez, 2021), we aimed to investigate whether cherries with different phenolic hallmarks show a seasonal response to TH and potential metabolic consequences on hepatic glucose metabolism.

As expected, animals increased BW over the 11 weeks as a physiological response of normal growing. Moreover, the control group exposed to the L6 photoperiod (L6-C group) showed a tendency to reduce BW compared to the L12-C group. Loss of BW and a decrease in food intake during a short photoperiod have been previously observed in F344 rats (Tavolaro et al., 2015). In addition, Mariné-Casadó et al., (2019) found that L18-exposed rats had a greater fat mass and a lower lean/fat mass ratio compared to L6 photoperiod-exposed rats. The L12 photoperiod was previously shown to induce a long-day physiology (Tavolaro et al., 2015). Regarding the effect of cherry on animals, similarly to our study, L18 and L6 photoperiod-exposed F344 rats consuming cherry (100 mg/kg BW) for 10 weeks, not produced effects

on BW in comparison with their respective control groups (Mariné-Casadó et al., 2019). In contrast, Ch1 consumption significantly reduced BW in the L6-Ch1 group compared with L12-Ch1 group in our study differently of Ch2 administration. Interestingly, these results suggest that Ch1 consumption could improve photoperiod signals for BW change whereas Ch2 would counteract the effect of photoperiod. Phenolic compounds contained in the cherries could be responsible for the observed effect on BW and the reason why both cherries showed a differential effect. Although cherry consumption had not been previously associated to this effect (Mariné-Casadó et al., 2019), there are evidence that different phenolic-rich extract from walnut (Shi et al., 2014) and *Alpinia galangal* (Kumar & Alagawadi, 2013), with *in vitro* inhibitory activity for pancreatic lipase, reduced BW (up to 40 %) of obese animals in comparison with control animals. Anti-obesity effects of phenolic compounds have been also observed in humans, reducing up to 3.48 kg the BW of obese volunteers consuming an isocaloric diet and supplemented with a phenolic-rich extract of *Lippia citriodora* and *Hibiscus sabdariffa* for 60 days (Boix-Castejón et al., 2018).

Given that TH (T3 and T4) availability have seasonally dependent production at the hypothalamic level (Bechtold & Loudon, 2007). In the present study, we investigated whether seasonal consumption of cherry could modulate the hypothalamus-pituitary-thyroid (HPT) axis, acting through TH output. Our results showed a photoperiod effect on circulating levels of both hormones (L6 > L12 = L18 for T3 and L6 = L12 > L18 for T4), indicating that the change in day length significantly altered TH levels in the periphery. In addition, administration of cherries to animals exerted different effects on serum T3 levels, as both cherries reverted the L6 effect observed in the L6-C group in a different manner, and L18-Ch2 group showed increased T3 levels with respect to the L18-C and L18-Ch1 groups; however, the tendency in all the cases was to set the TH levels equal to L12-C, tending to counteract the photoperiod effect. Several studies have reported that (poly)phenols can bind to several enzymatic activities related to thyroid function, including thyroperoxidase (TPO) inhibition (de Souza dos Santos et al., 2011). This enzyme facilitates the addition of iodine to thyroglobulin, which is crucial for the TH generation (Billek et al., 2020). However, more recently, it has been observed that certain phenolic compounds such as ferulic, *trans*-cinnamic, and syringic acids exert a contrary effect on TPO, producing its activation in a dose-dependent manner (Habza-Kowalska, Kaczor, Bartuzi, Piłat, & Gawlik-Dziki, 2021). Thus, the differential effects of the consumption of Ch1 and Ch2 could be attributed to the sum of the inhibitory and activating signals on TH synthesis produced by their different profiles and concentrations of different phenolic compounds. Another factor that should be considered is the mineral composition of the fruit. The major mineral found in cherries is potassium, followed by phosphorus, calcium, and magnesium (ten times lower). However, they can also contain certain levels of minerals such as iodine, selenium or iron that, although present in low amounts (<1 mg/100 g edible portion) (Serradilla et al., 2015), are elements that demonstrated to be crucial for TH synthesis and metabolism (Köhrle, 2023).

One of the target organs of TH is the liver, which is finely regulated by multiple nutrients, hormones, and neuronal signals, which in turn regulate glucose and lipid metabolism at the hepatic level (Brent, 2012; Lin & Sun, 2011). Thus, the potential role of TH as a key mediator of the metabolic hepatic effect and the effect of cherry consumption on it was investigated. First, hepatic *Dio1* expression was quantified, as a marker of T3 status at the hepatic level (Zavacki et al., 2005). DIO1 is responsible for the activation of T4 in T3 in the liver (Gereben et al., 2008). Our results showed that it is upregulated during a short photoperiod in the C group, indicating that T4 conversion in the active form is regulated in a photoperiod-dependent manner.

In addition, genes involved in glucose metabolism were studied, starting with *Glut2*. GLUT2 mediates the uptake and release of glucose in hepatocytes (Karim et al., 2012). No effects of photoperiod were observed in this gene; however, gene expression was resulted significantly different between Ch1 and Ch2 under a short photoperiod. According to these results, it seems that *Glut2* was not modulated by TH, given that a photoperiod effect was observed in both T3/T4 blood levels and hepatic *Dio1* gene expression. Our results do not seem to agree with a previous *in vivo* study that observed an increase in GLUT2 and *Glut2* expression levels in the liver of hyperthyroid rats compared to euthyroid and hypothyroid rats (Weinstein, Oboyle, Fisher, & Haber, 1994). Considering that the deletion of *Glut2* at the hepatic level does not compromise hepatic glucose production in the fasting state (Seyer et al., 2013), we examined the possible activation of key gluconeogenic enzymes by TH. In this regard, it has been reported that TH regulate gluconeogenesis in the liver, directly upregulating TH target genes such as the gluconeogenic transcription factor *Forkhead Box O1* (*Foxo1*) and key gluconeogenic enzymes (*Pck1* and *G6pc*) in the liver (Sinha et al., 2014). During a long photoperiod, downregulation of *Pck1* and *G6pc* expression was observed, which was slightly reversed by Ch2 consumption in both the cases. This could be attributed to the lower concentration of T3 in the blood found in the L18-C group than in the L6-C group. A photoperiod effect was also observed in liver *Pck1* (not *G6pc*) expression in F344 rats exposed to L6, L12, and L18 photoperiods for 14 weeks (longer than in our study); however, they observed an upregulation of this gene in the L12 group with respect to the L6 and L18 groups (Mariné-Casadó et al., 2018). Previous studies have already shown that cherry consumption acts on hepatic metabolism regulation, specifically regulating the gene expression of the fatty acid synthase and sterol regulatory element binding protein 1 (lipogenic enzymes) in a photoperiod-dependent manner (Ruiz de Azua, Cruz-Carrión, Muguerza, Arola-Arnal, & Suarez, 2021).

In the present study, *Nampt* gene expression was also investigated as NAMPT is the rate-limiting enzyme of NAD^+ production. NAD is essential for the oxidation and reduction reactions and signalling pathways (Borradaile et al., 2011). We found that this gene was upregulated in all groups during long photoperiod compared with short and intermediate photoperiods, which is not in concordance with the *Pck1* and *G6pc* expression results. These results are not in agreement with those previously observed by Mariné-Casadó et al., (2019), where no statistical effects were observed in this gene by photoperiod, although expression levels in the long photoperiod group were higher than in the short and intermediate groups. In addition, in our study a significant downregulation was observed in L18 animals consuming Ch2, which counteracted the L18 photoperiod effect observed in the L18-C group, while Ch1 administration did not produce any effects on this gene. Neither liver nor muscle *Nampt* expression was altered in L6 and L18 photoperiod-exposed Fisher 344 rats supplemented with sweet cherry for the last 10 weeks of the study (Mariné-Casadó et al., 2019). However, changes in hepatic and hypothalamic *Nampt* expression have been observed following the consumption of grape seed proanthocyanidin extract in Wistar rats (Ribas-Latre et al., 2015). Moreover, a dose-dependent increase in hepatic NAD^+ was observed in Wistar rats consuming grape seed proanthocyanidin extract for 21 days at different doses (up to 50 mg/kg BW) (Aragonès et al., 2016). As mentioned above, our previous studies showed that cherries with different phenolic compositions could exert different effects on hepatic oxidative stress (Cruz-Carrión et al., 2020) and lipogenic enzyme gene expression (Ruiz de Azua, Cruz-Carrión, Muguerza, Arola-Arnal, & Suarez, 2021) and neuroprotection in the hippocampus (Manocchio, Bravo, Helfer, & Muguerza, 2024). Indeed, Ch1 exhibited higher levels of total polyphenols, anthocyanins, and flavanols than Ch2 (Cruz-Carrión et al., 2020). Conversely, Ch2 contained higher levels of total flavonols than Ch1 did. In addition, anthocyanins were the main phenolic family in both cherries and for individual compounds cyanidin-3-*O*-rutinoside (more abundant in Ch1), quercetin-3-O-rutinoside (more abundant in Ch2), catechin (more abundant in Ch1), 4-hydroxybenzoic acid (more abundant in Ch2), and 3-*O*-caffeoylquinic acid (more abundant in Ch1) were the most abundant when analysed by HPLC-ESI-MS/MS (data unpublished). Thus, the different phenolic profiles of both tested

cherries could explain the differences in liver *Nampt* expression between them. *Gk* levels were upregulated during L18 photoperiod, but no differences were observed when the rats consumed Ch1 or Ch2. These results suggest that hepatic gluconeogenesis is downregulated under long photoperiod exposure and can be reversed by the consumption of cherries with a specific phenolic hallmark.

Finally, gluconeogenesis-related metabolites, such as alanine, valine, and glycerol, were analysed in the serum to further investigate the crosstalk between TH and glucose metabolism. ^{1}H NMR-based metabolomic profiles indicated that the Cori cycle and triglyceride hydrolysis might be upregulated in the C group with respect to the Ch1 group during a short photoperiod, explaining the higher levels of lactate and glycerol. However, further studies investigating muscles metabolism are required to elucidate the exact meaning and impact of these results. Moreover, as suggested by hepatic gene expression, the downregulation of gluconeogenesis under a long photoperiod is supported by a decrease in circulating glucogenic amino acids, such as valine and alanine, with respect to a short photoperiod. Here, we confirmed that the metabolome profile is modulated in a photoperiod-dependent manner. Indeed, as has been previously observed, the adaptation to seasonal variation in humans, who live far from the equator, showed an increased blood levels of cholesterol, triacylglyceride, glucose, leptin, and insulin during winter (Kanikowska et al., 2015; Ockene et al., 2004).

5. Conclusions

The results showed that sweet cherries can modulate TH levels depending on both photoperiod and geographical origin. Moreover, TH metabolic outcomes could be related to hepatic gluconeogenesis in a photoperiod- and cherry-type-dependent manner. Specifically, Ch1 showed effects mostly during a short photoperiod, while Ch2 was mainly during a long photoperiod, tending to minimize the differences between photoperiods. Further research is needed to elucidate the crosstalk between the consumption of fruits rich in phenolic compounds and the photoperiodic peripheral modulation of metabolic pathways.

Ethics Statement

The animal protocols were 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 the Generalitat de Catalunya (permission number 9495, FUE-2017–00499873).

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CRediT authorship contribution statement

Francesca Manocchio: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **María Josefina Ruiz de Azua:** Methodology, Investigation. **Èlia Navarro-Masip:** Methodology, Investigation. **Diego Morales:** Writing – review & editing, Data curation. **Gerard Aragonès:** Funding acquisition. **Cristina Torres-Fuentes:** Funding acquisition. **Begoña Muguerza:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Francisca Isabel Bravo:** Writing – review & editing, Supervision, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2024.106415.

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