RESEARCH ARTICLE

Molecular Nutrition
Food Research
www.mnf-journal.com

Vitis vinifera L. Bioactive Components Modulate Adipose Tissue Metabolic Markers of Healthy Rats in a Photoperiod-Dependent Manner

*Èlia Navarro-Masip, Francesca Manocchio, Marina Colom-Pellicer, Xavier Escoté, Lisard Iglesias-Carres, Enrique Calvo, Francisca I Bravo, Begoña Muguerza, Yves Desjardins, and Gerard Aragonès**

Scope: The beneficial health effects of (poly)phenol-rich foods such as red grapes mainly depend on both the type and concentration of (poly)phenols. Since fruit (poly)phenol content is influenced by growing conditions, the study examines the seasonal effects of red grapes (*Vitis vinifera* L.), grown under various cultivation conditions, on metabolic markers of adipose tissue in healthy rats.

Methods and results: For this purpose, Fischer 344 rats are exposed into three different light-dark cycles and daily supplemented with 100 mg kg^{-1} of either conventionally or organically grown red grapes for 10 weeks ($n = 6$). Seasonal consumption of organic grapes (OGs), which are richer in anthocyanins, increases energy expenditure (EE) of animals exposed to long photoperiod and enhances uncoupling protein 1 (UCP1) protein expression in brown adipose tissue of animals under standard photoperiod. Additionally, red grape consumption affects the gene expression profile of white adipose tissue (WAT), upregulating browning markers of subcutaneous WAT in 12 h light (L12) and 18 h light (L18) photoperiods, and downregulating adipogenic and lipolytic markers of visceral WAT in 6 h light (L6) and L12 photoperiods.

Conclusions: These results clearly show that bioactive compounds of grapes can modulate the metabolic markers of white and brown adipose tissues in a photoperiod and depot-dependent manner, partly affecting EE when consumed out of season.

1. Introduction

Fruit consumption is one of the main elements of a healthy diet and lifestyle. The main benefits of fruits come from their richness in micronutrients and bioactive components, such as (poly)phenols.[1] Grapes are a great example of (poly)phenol-rich fruits, containing mainly flavan-3-ols, anthocyanins, and phenolic acids.[2] These phenolic compounds have been widely associated with protection against metabolic pathologies such as cardiovascular disease and obesity.[3] Additionally, it has been observed that (poly)phenol intake may confer environmental adaptative advantages to heterotrophs providing metabolic cues about stressful conditions.[4]

Once ingested, (poly)phenols are subjected to extensive microbial catabolism and phase II metabolism by the enterocytes and the liver. It is now widely accepted that these metabolites are responsible for the beneficial effects of (poly)phenols.[5,6] Interestingly, bioavailability and metabolism of red grape

È. Navarro-Masip, F. Manocchio, M. Colom-Pellicer, L. Iglesias-Carres, E. Calvo, F. I Bravo, B. Muguerza, G. Aragonès
Nutrigenomics Research Group, Department of Biochemistry and Biotechnology
Universitat Rovira i Virgili
Tarragona 43007, Spain
E-mail: gerard.aragones@urv.cat

X. Escoté
Unitat de Nutrició i Salut
Centre Tecnològic de Catalunya
Eurecat 43204, Reus, Spain

E. Calvo, F. I Bravo, B. Muguerza, G. Aragonès
Institute of Health Pere Virgili (IISPV)
Tarragona 43007, Spain

E. Calvo, F. I Bravo, B. Muguerza, G. Aragonès
Center of Environmental, Food and Toxicological Technology (TecnATox)
Universitat Rovira i Virgili
Tarragona 43007, Spain

Y. Desjardins
Faculty of Agriculture and Food Sciences
Institute of Nutrition and Functional Foods (INAF)
Laval University
Québec, QC G1V 0A6, Canada

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/mnfr.202300074

DOI: 10.1002/mnfr.202300074

(poly)phenols depend on how fruits are grown, as previously demonstrated in both healthy and obese rats.[7] In particular, grapes cultivated under organic conditions (organic grape [OG]) contained lower amounts of dietary fiber than those grown under conventional conditions (conventional grape [CG]), resulting in higher bioaccessibility of total (poly)phenols. Additionally, OG also contained 40% higher amounts of anthocyanins, while CG had higher concentrations of free catechins and some specific flavonols. Notably, the bioaccessibility of (poly)phenols was significantly increased when rats raised under winter-simulatedind photoperiod (rats exposed to short-day photoperiod of 6-h light:18-h dark cycles) were fed red grapes.[8] Thus, (poly)phenol-rich fruits influence the seasonal metabolic adaptations of mammals to their environment.[9] Factors affecting the (poly)phenol content of fruits, such as growing conditions, may thus influence their effect as metabolic cues. Moreover, white adipose tissue (WAT) can efficiently adapt energy metabolism according to seasonal requirements.[10] In fact, the morphology and activity of WAT and of brown adipose tissue (BAT) change through the seasons of the year[11] to sustain whole-body energy homeostasis.

Our group has demonstrated that photoperiod can affect (poly)phenol bioavailability. Additionally, these molecules act as dietary cues affecting the response of adipose tissue to photoperiod, that is, they promote lipid accumulation in adipocytes when they are consumed out of season.[12,13] Indeed, animals need to adapt their physiology to cold conditions and red grapes (poly)phenol consumption can play a role in adaptations to winter. It is proposed that this seasonal response to grape consumption is mediated by hypothalamic leptin signals in both normal-weight and obese rats.[14] Moreover, the expression profile of lipogenic genes in the liver was also affected by seasonal consumption of (poly)phenol-rich fruits, reinforcing the signaling effects of these compounds on lipid metabolism.[15] Considering the seasonal-dependent impact of (poly)phenol consumption on lipid metabolism, we focused this work on the effects of seasonal consumption of grapes containing different bioactive compound profiles on markers of energy metabolism in WAT and BAT of healthy rats. Therefore, the aim of this study is to assess the effects of the consumption of *Vitis vinifera* L. with different (poly)phenol content, as affected by the growing conditions (conventional or organic), on the metabolic markers of different fat depots in healthy Fischer 344 rats exposed to different photoperiods.

2. Results

2.1. Red Grapes Grown under Organic Conditions Contained Higher Amounts of Anthocyanins

The differences in (poly)phenol composition of red grapes cultivated under different conditions were studied; OG contained significantly more total phenolic compounds than their CG counterpart (Supplementary Figure S2). Particularly, we observed a 47% increase in the anthocyanin content for OG, compared to CG, constituted mainly of malvidin-3-*O*-glucoside. On the other hand, CG had slightly higher amounts of flavonols and flavanols.

2.2. Consumption of OG Resulted in a Reduced Food Intake in Animals Subjected to L6 Photoperiod

When a two-way analysis of variance (ANOVA) test was conducted, we could not identify any interactions between photoperiod and red grape (poly)phenols consumed. We could only observe a significant effect of photoperiod (represented in **Table 1** as *P* effect) on different metabolic parameters including body weight, food intake, respiratory quotient (RQ), and adipose tissue weight, which were reduced in animals exposed to L6 photoperiod. Similarly, serum triglyceride (TG) concentrations were reduced in L12 photoperiod groups (Table 1). Notably, although no significant changes were observed in body weight and fat content in response to grape administration, OG consumption, but not CG, significantly reduced accumulated caloric intake in animals subjected to L6 photoperiod compared to rats consuming vehicle (VH) in the same photoperiod ($p = 0.014$). Additionally, OG consumption also reduced serum glucose levels compared to CG consumption in animals subjected to L12 photoperiod. Yet, a grape-dependent increase in serum glucose concentration was observed in animals exposed at L6 photoperiod ($p = 0.016$), while serum TG and total cholesterol concentrations were not affected in response to fruit consumption.

2.3. Consumption of CG and OG Enhanced EE in Animals Exposed to L18 and L12 Photoperiods

As shown in **Figure 1**, consumption of grapes did not significantly affect energy expenditure (EE) in animals exposed to L6 photoperiod (Figure 1A). However, the area under the curve (AUC) was significantly reduced in response to grape consumption in animals exposed to L6 photoperiod. Nevertheless, both CG and OG supplementation significantly increased EE in animals exposed to 18 h light photoperiod (L18) and L12 photoperiods (Figure 1B,C). Additionally, changes in locomotor activity were in concordance with EE values, although these changes did not reach statistical significance (Figure 1D–F).

2.4. OG Consumption Significantly Upregulated Thermogenic Genes in BAT in Animals Exposed to L12 Photoperiod

Then, the key molecular factors responsible for BAT functionality were analyzed, considering that this tissue could be likely involved in the EE adaptations observed in response to fruit consumption. Two-way ANOVA analyses revealed a significant interaction effect between fruit consumption and photoperiod on mRNA levels of most of the genes involved in thermogenesis (uncoupling Protein 1 [*Ucp1*], deiodinase 2 [*Dio2*], carnitine palmitoyltransferase I beta [*Cpt1β*], peroxisome proliferator-activated receptor gamma coactivator 1 alpha [*Pgc1α*]), brown adipogenesis (PR domain containing 16 [*Prdm16*], peroxisome proliferator activated receptor gamma [*Pparγ*], peroxisome proliferator activated receptor alpha [*Pparα*]), and lipid transport (lipoprotein lipase [*Lpl*]). To assess the specific effects of CG and OG consumption under each photoperiod, we conducted one-way ANOVA analyses to compare the distinct groups under one same photoperiod. In L6 photoperiod, *Pparα* and *Pparγ*

Table 1. Biometric and serum parameters.

	Photoperiod	VH	CG	OG	Two-way ANOVA
Body weight [g]	L6^{2}	365.50 ± 9.5	361.00 ± 7.1	362.67 ± 10.7	*P*
	L12	377.83 ± 6.4	382.00 ± 8.5	383.00 ± 12.0	
	L18	384.17 ± 13.2	376.83 ± 6.1	391.33 ± 13.5	
Body weight gain [g]	L6	190.54 ± 7.7	178.51 ± 9.1	184.83 ± 10.5	*NS*
	L12	180.01 ± 6.7	180.17 ± 7.8	184.03 ± 10.3	
	L18	191.58 ± 8.3	187.50 ± 6.1	205.83 ± 13.0	
Accumulated caloric intake [kcal]	L6^{2}	634.79 ± 10.2^{a}	604.98 ± 7.0^{a,b}	592.68 ± 10.0^{b}	*P*
	L12	631.7 ± 9.7	647.8 ± 7.3	640.19 ± 5.5	
	L18	625.76 ± 12.8	614.91 ± 3.7	626.92 ± 18.6	
24 h RQ	L6^{2}	0.81 ± 0.01	0.81 ± 0.01	0.79 ± 0.02	*P*
	L12	0.84 ± 0.02	0.87 ± 0.01	0.85 ± 0.01	
	L18	0.86 ± 0.01	0.86 ± 0.01	0.85 ± 0.01	
Fat mass [g]	L6	45.06 ± 1.3	51.18 ± 3.1	50.30 ± 3.2	*NS*
	L12	52.71 ± 3.7	47.51 ± 2.2	50.79 ± 3.4	
	L18	55.64 ± 4.4	54.35 ± 4.7	56.10 ± 6.2	
Lean mass [g]	L6^{2}	295.76 ± 8.2	287.95 ± 7.3	286.98 ± 7.3	*P*
	L12	307.84 ± 5.7	315.31 ± 8.7	313.66 ± 8.0	
	L18	309.74 ± 9.0	303.65 ± 6.02	314.23 ± 10.6	
BAT mass [g]	L6	0.33 ± 0.07	0.26 ± 0.03	0.23 ± 0.03	*P*
	L12	0.31 ± 0.04	0.33 ± 0.03	0.36 ± 0.01	
	L18	0.36 ± 0.04	0.37 ± 0.05	0.36 ± 0.04	
iWAT mass [g]	L6^{2}	4.52 ± 0.5	4.50 ± 0.5	4.47 ± 0.4	*P*
	L12	5.47 ± 0.4	5.27 ± 0.5	6.38 ± 1.3	
	L18	5.82 ± 0.7	6.02 ± 0.8	5.88 ± 0.6	
rWAT mass [g]	L6	8.52 ± 0.6	8.38 ± 0.4	8.92 ± 0.6	*P*
	L12	9.57 ± 0.5	9.25 ± 0.4	9.49 ± 0.6	
	L18	10.00 ± 0.7	10.12 ± 0.7	10.49 ± 1.0	
Glucose [mmol L^{-1}]	L6	6.04 ± 0.9^{a}	8.53 ± 2.1^{b}	7.91 ± 0.6^{b}	*P*
	L12	5.42 ± 1.6^{a,b,}	6.11 ± 0.9^{a}	5.16 ± 0.5^{b}	
	L18	5.61 ± 1.6	5.49 ± 1	5.76 ± 2.1	
TG [mmol L^{-1}]	L6	1.39 ± 0.3	1.59 ± 0.5	1.21 ± 0.2	*P*
	L12^{2}	0.81 ± 0.2	1.11 ± 0.2	1.16 ± 0.5	
	L18	1.46 ± 0.3	1.26 ± 0.5	1.34 ± 0.2	
Cholesterol [mmol L^{-1}]	L6	3.62 ± 1.2	3.18 ± 0.5	3.56 ± 0.5	*NS*
	L12	3.02 ± 1.1	3.69 ± 1.1	2.82 ± 0.9	
	L18	3.12 ± 0.4	2.91 ± 0.4	3.36 ± 0.4	

Values are presented as the mean ± SEM of six animals per group. Different letters denote significant changes assessed by one-way ANOVA and Tukey's post-hoc test ($p < 0.05$). ANOVA, analysis of variance; BAT, brown adipose tissue; CG, conventional grape; iWAT, inguinal white adipose tissue; NS, not significant; OG, organic grape; P, photoperiod effect ($p < 0.005$); RQ, respiratory quotient; rWAT, retroperitoneal white adipose tissue; SEM, standard error of the mean; TG, triglyceride; VH, vehicle.

gene expression was significantly downregulated in response to fruit consumption, while *Ucp1* and *Dio2* gene expression was downregulated only in response to OG consumption (**Figure 2**A). Nonetheless, when CG was consumed in animals exposed to L12 photoperiod, a significant downregulation in the expression of most of the genes studied was observed, including *Ucp1*, *Dio2*, *Pgc1α*, *Cpt1β*, *Pparγ*, and fatty acid transport protein 1 (*Fatp1*, Figure 2A–C). Interestingly, OG consumption completely reverted this effect, yet upregulating *Prdm16* and cluster of differentiation 36 (*Cd36*) gene expression. In contrast, in L18 animals, CG and OG consumption resulted in an upregulation of *Pgc1α* gene expression, and only CG consumption caused an upregulation of two important lipid transport-related genes (*Cd36* and *Fatp1*) compared to VH group (Figure 2C). Noteworthy, Western blot assays showed that fruit consumption did not affect UCP1 protein expression in L6 animals (Figure 2D), while OG consumption tended to decrease it in L18 compared to CG consumption. In addition, the immunoblots confirmed higher levels of UCP1 protein expression in response to OG consumption only when animals were exposed to L12 photoperiod with respect to both VH and CG groups.

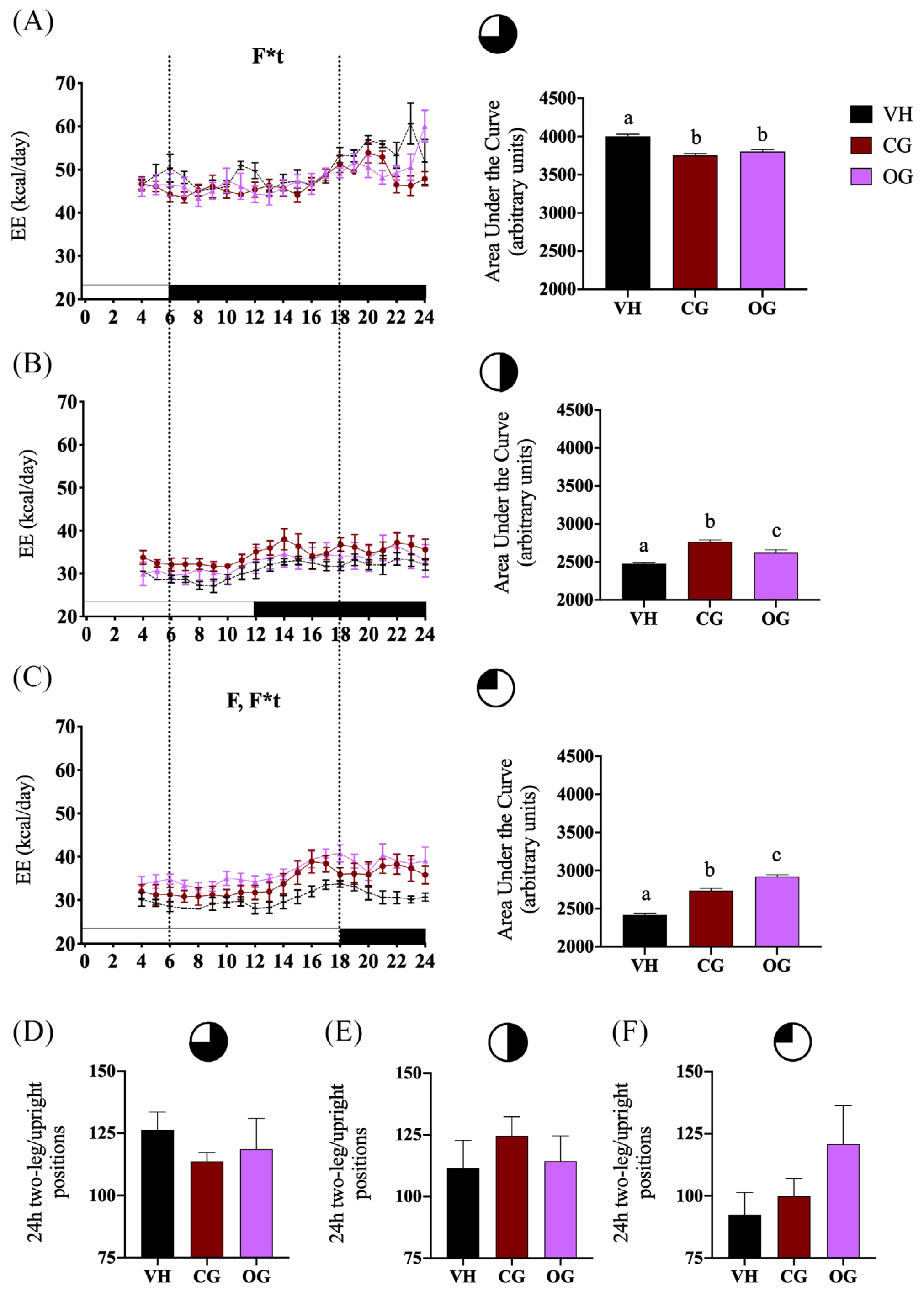
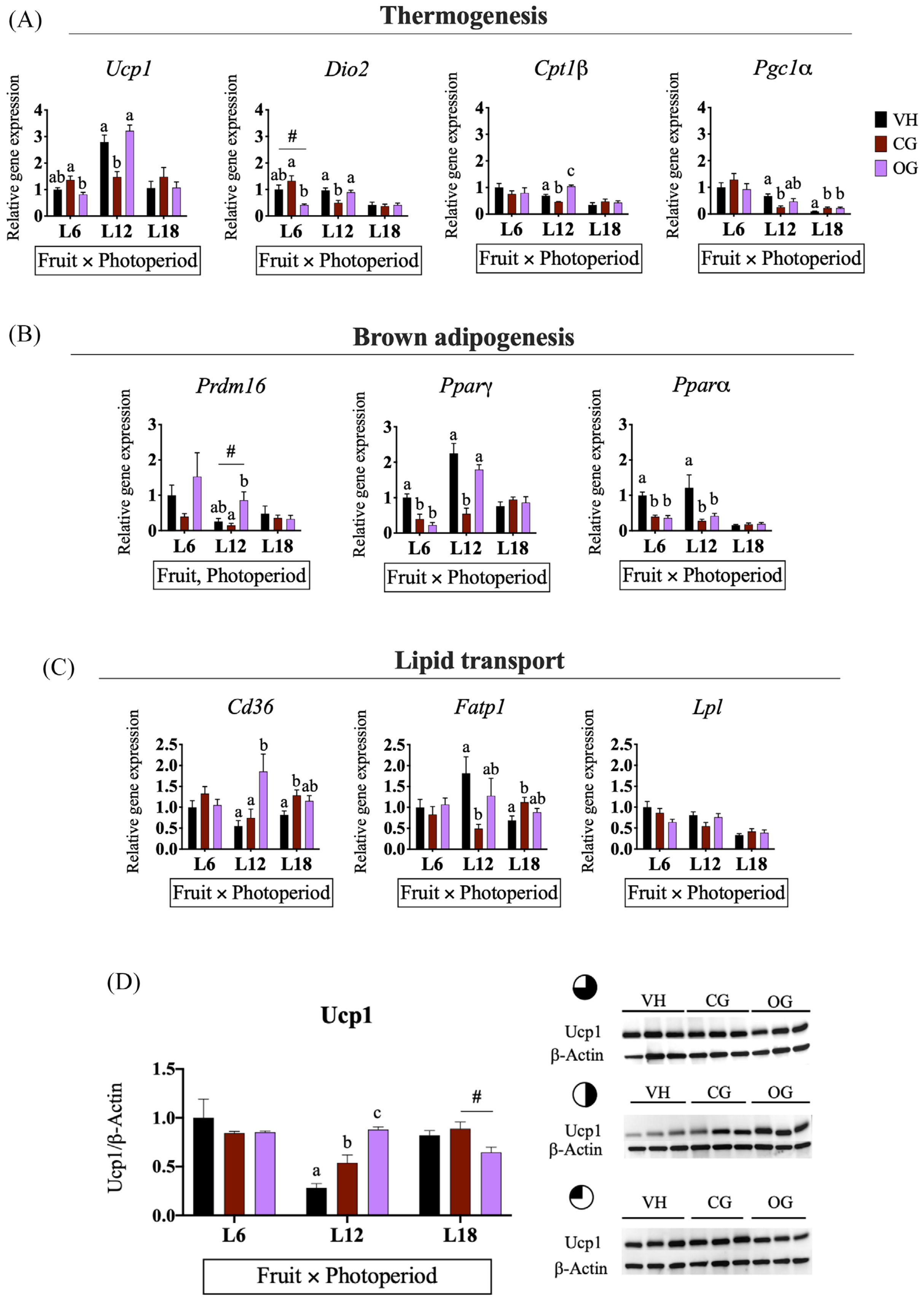


Figure 1. Effect of seasonal consumption of conventional (CG) or organic grape (OG) on energy expenditure (EE). Twenty hours of indirect calorimetry were measured in animals fed a standard chow diet + vehicle or 100 g kg^{-1} of lyophilized CG or OG for a 10-week-period and adapted to 6 h light per day (L6, a), 12 h light per day (L12,b), or 18 h light per day (L18, c) photoperiod. Values are mean ± standard error of the mean (SEM) ($n = 6$). *F*, fruit effect; *t*, time effect; $F^{*}t$, interaction of fruit treatment and time, assessed by two-way analysis of variance (ANOVA, $p < 0.05$). Area under the curve is calculated for the data of groups for each photoperiod, and motor activity was described by the total amount of two-leg/upright positions during 24 h (L6, d; L12, e; L18, f). Statistical significance was assessed by one-way ANOVA followed by Tukey's post-hoc test.


(A)
Thermogenesis
Ucp1
Dio2
Cpt1β
Pgc1α
Relative gene expression
0
1
2
3
4
L6
L12
L18
ab
a
b
#
Fruit × Photoperiod
VH
CG
OG
(B)
Brown adipogenesis
Prdm16
Pparγ
Pparα
Fruit, Photoperiod
(C)
Lipid transport
Cd36
Fatp1
Lpl
0.0
0.5
1.0
1.5
2.0
2.5
(D)
Ucp1
Ucp1/β-Actin
c
VH
CG
OG
Ucp1
β-Actin

2.5. Consumption of CG and OG Upregulated Subcutaneous *Ucp1* Gene Expression in Animals Exposed to L18 and L12 Photoperiods

Considering the adaptations in EE in L12 and L18 in response to fruit consumption, we surmised that WAT should also be affected at the molecular level despite finding no differences in body weight, as it has been previously demonstrated.[16,17] Thus, we assessed the metabolic gene expression profile of several markers of lipid metabolism in inguinal white adipose tissue (iWAT) and retroperitoneal white adipose tissue (rWAT), representing subcutaneous and visceral white adipose profiles, respectively. Browning markers were also determined in iWAT, the adipose region with higher browning susceptibility.

In iWAT (**Figure 3**), we observed a significant fruit type effect on the gene expression of adipogenic markers (*Pparγ* and CCAAT-enhancer-binding protein alpha [*C/ebpα*]) glycerol-3-phosphate acyltransferase (*Gpat*) and *Lep*. A significant effect of photoperiod was also observed on lipolytic (hormone sensitive lipase [*Hsl*]), lipogenic (*Gpat*, fatty acid synthase [*Fasn*], acetyl-CoA carboxylase 1 alpha [*Acacα*]), and adiponectin (*Adipoq*) gene expression. An interaction effect was only observed in *Ucp1* and *Pgc1α* gene expression (Figure 3D). After applying one-way ANOVA analyses, we observed that, under L12 photoperiod, CG consumption upregulated the expression of the adipogenic genes *Pparγ* and *C/ebpα* as well as the expression of an important lipolysis-related gene (adipose triglyceride lipase [*Atgl*]). In addition, both types of grapes upregulated *Lep* gene expression, and only OG upregulated *Ucp1* mRNA levels. In L18 groups, *Fasn* was downregulated by consumption of both CG and OG, although *Acacα* and *Adipoq* gene expression was only upregulated in response to OG consumption. In a similar fashion to L12 photoperiod, both types of grapes upregulated *Ucp1* gene expression under L18 photoperiod.

In rWAT, in contrast to iWAT, we observed a significant effect of fruit type on most of the metabolic genes we analyzed, showing a general downregulation of their expression in response to both OG and CG consumption (**Figure 4**). Additionally, a photoperiod effect was detected in the expression of most of these genes, except for *Adipoq*. When we applied one-way ANOVA analyses, we detected that both types of grapes significantly downregulated the gene expression of *Pparγ*, *Cebpα*, *Atgl*, *Hsl Cd36*, and *Adipoq* in animals subjected to L6 photoperiod. Similar results were observed in L12 groups, where CG and OG downregulated the gene expression of *Pparγ*, *Hsl*, *Cd36*, and *Adipoq*, whereas *Cebpα* was only modulated by CG consumption. In animals subjected to L18 photoperiod, *Pparγ* and *Lep* gene expressions were again modulated only in response to CG, whereas OG consumption significantly downregulated *Cd36* gene expression.

Overall, we observed that both fruit consumption and photoperiod exposure altered the expression of the main metabolic genes in the iWAT and rWAT. There was an interaction effect between the two variables for only a few genes. Moreover, the distinct types of grapes differentially modulated each adipose depot. The expression of metabolic genes in rWAT was particularly affected by fruit consumption in L6 animals, while little effects were detected on iWAT under this photoperiod. Globally, fruit consumption reduced the expression of most of the genes that we assessed in the visceral adipose depot.

3. Discussion

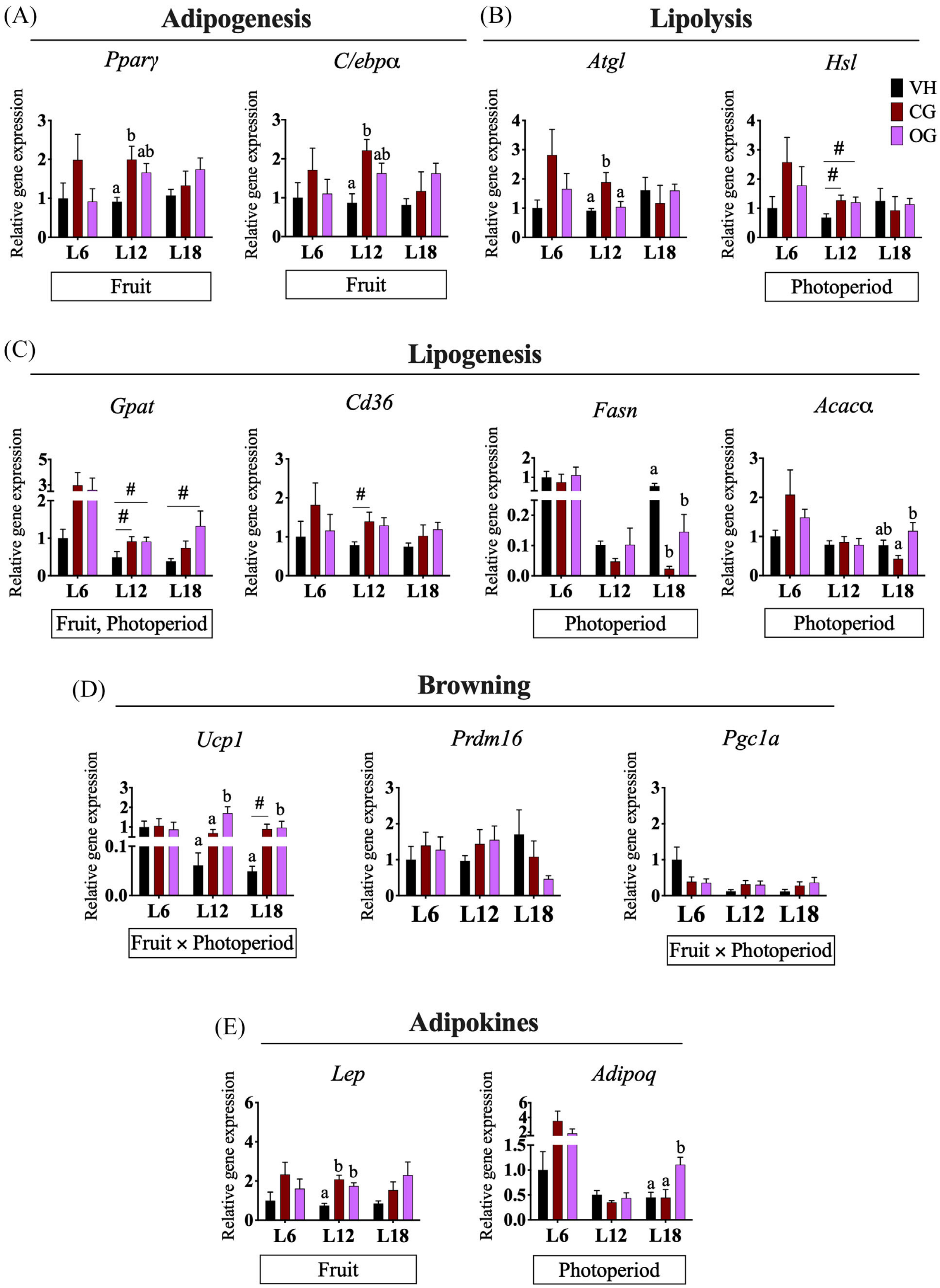
Our study aimed to elucidate the effect of the consumption of grapes containing different content of bioactive compounds, resulting from different growing methods, on the lipid metabolism of adipose tissues and other associated metabolic markers. In particular, OG fruits accumulated significantly more phenolic compounds, and especially anthocyanins, as a result of their exposure to higher biotic and abiotic stresses.[7,8,18] Therefore, we hypothesized that these differences in phenolic composition of grapes could influence the molecular response of rats exposed to different photoperiods, simulating the distinct seasons of the year.

It is now firmly established that photoperiod influences metabolism. Indeed, many human studies have demonstrated that seasonality clearly affects body mass and adiposity, with an increase in body weight under longer photoperiods.[19,20] This metabolic adaptation to daylength was also observed in animal models,[17,21] and in Fischer 344 rats which are especially suited to metabolic adaptations in response to different photoperiods.[22]

Our results clearly reflect the impact of (poly)phenol content of grapes, as affected by cultivation conditions, on the energy metabolism of rats. First, animals that consumed OG during the natural season of grapes (L6 photoperiod) had a lower caloric intake than L12 and L18. It should be noted that the traditional consumption of grapes is occurring in winter, where the photoperiod is short.[8] Additionally, RQ values were lower in all L6 animals and, in particular, in response to OG consumption, as we demonstrated in previous studies using similar conditions.[14,23] In fact, EE during L6 was greatly increased, confirming the strong impact of photoperiod on metabolism. Indeed, Fischer 344 rats tended to gain weight under summer-like conditions (i.e., L18), while losing weight under winter-like conditions (i.e., L6).[24–26] Our results support this observation, as photoperiod affected total body weight; animals exposed to L18 photoperiod had higher body weight compared than those exposed to L6.

Interestingly, consumption of both types of grapes increased EE of rats exposed to L18 photoperiod. As expected, rats, being nocturnal animals, also showed higher EE under longer dark periods. It is interesting to speculate that this response to (poly)phenols may represent a dietary cue, whereby animals consuming (poly)phenols from grapes during the summer-like

Figure 2. Effect of seasonal consumption of conventional (CG) or organic grape (OG) on brown adipose tissue (BAT) activity. Expression of genes related to thermogenesis (A), adipogenesis (B), and lipid transport (C) was evaluated by quantitative PCR and protein expression of uncoupling protein 1 (UCP1) (D) was quantified by Western blot analysis, on animals fed standard chow diet + 100 mg kg^{-1} of lyophilized CG or OG for 10-week-period and submitted into short day (L6), standard (L12), or long day (L18) photoperiods. Values were normalized against vehicle group of L6 photoperiod. Data represent mean ± standard error of the mean (SEM) ($n = 5$–6). *Fruit*: fruit effect; *Photoperiod*: photoperiod effect; *Fruit × Photoperiod*: interaction of photoperiod and fruit treatment assessed by two-way analysis of variance (ANOVA, $p < 0.05$). Different letters denote effect of fruit on each photoperiod group determined by one-way ANOVA and Tukey's post-hoc test ($p < 0.05$).


(A)
Adipogenesis
Pparγ
C/ebpα
Relative gene expression
0
1
2
3
L6
L12
L18
a
b
ab
Fruit
Fruit
(B)
Lipolysis
Atgl
Hsl
4
VH
CG
OG
#
#
Photoperiod
(C)
Lipogenesis
Gpat
Cd36
Fasn
Acacα
5
0.0
0.1
0.2
Fruit, Photoperiod
Photoperiod
Photoperiod
(D)
Browning
Ucp1
Prdm16
Pgc1a
Fruit × Photoperiod
Fruit × Photoperiod
(E)
Adipokines
Lep
Adipoq
6
0.5
1.0
1.5
Fruit
Photoperiod

photoperiod metabolically adapt to face the winter environmental conditions, as grapes are set to be available for consumption in this season. Otherwise, EE was diminished after grape consumption when animals were exposed to the short photoperiod. These animals also showed lower caloric intake, but its body weight was unchanged, compared to the VH-L6 group. Therefore, in-season grape consumption promoted a reduction of both food intake and EE, while maintaining energy balance in these animals. Surprisingly, these grape-dependent changes in EE were not associated with changes in motor activity, therefore other mechanisms may thus be involved.

BAT is a key thermogenic tissue in rodents and plays a central role in fostering changes in energy metabolism. However, previous report revealed that BAT activity was diminished under short photoperiod in Fischer 344 rats.[17] In our study, we also observed a reduction in BAT weight in rats subjected to L6 photoperiod, and their gene expression levels were also downregulated compared to L12 animals. However, results concerning changes in metabolic BAT markers after grape consumption were perplexing, since the most significant differences were observed in the L12 group, representing fall/spring photoperiod. Surprisingly, we observed opposite effects after the consumption of the different types of grapes. While CG consumption significantly reduced the expression of thermogenic and adipogenic genes in BAT, OG not only reversed these effects at mRNA level, but it also significantly boosted UCP1 protein expression, an uncoupler of mitochondrial proton motive force. The presence of UCP1 in BAT is key to understand its activity status, since it confers to brown-like adipocytes their main thermogenic function, that is, heat generation from the mitochondrial fatty acid oxidation chain.[27] In our study, the 1.6 higher amounts of anthocyanidins in OG, compared to CG,[7] could account for the activation of BAT after OG consumption. Moreover, rats consuming OG showed higher serum concentrations of flavan-3-ol derived metabolites, phenolic compounds that enhance BAT activity.[28–31] Additionally, different studies reported that BAT activity was enhanced by cyanidin-3-glucoside, an anthocyanin capable of increasing EE through BAT activation and browning in mice.[32,33] Therefore, one can speculate that the higher content of these bioactive compounds in OG is associated with a higher expression of BAT markers in L12 rats, compared to rats consuming CG. Nevertheless, this pattern was not observed in the other two photoperiods, suggesting that the effects of grape (poly)phenols on BAT are photoperiod dependent. In fact, both types of grapes reduced UCP1 expression in BAT under L6, which could be an explanation to the reduced EE observed in these animals. Still, it may be interesting to further explore the impact of the different classes of (poly)phenols on the differentiation of adipose tissue in future experiments.

In animals subjected to L18 photoperiod, *Pgc1α* mRNA was upregulated in BAT after consumption of both types of grapes. This transcription factor is directly involved in brown adipogenesis and thermogenesis.[34] However, taking into account that UCP1 expression was downregulated by OG, this response suggests that BAT activity was probably not involved in the observed changes in EE. Other factors affecting energy metabolism could explain this surge in EE resulting from grape consumption in the L18 group. Indeed, the effect of (poly)phenols in the organism indirectly involves bile acid metabolism, which can impact EE through the action of thyroid hormone system in the skeletal muscle.[35,36]

After analyzing the seasonal effects of CG and OG consumption in the WAT, we clearly observed an influence of their specific composition in animals raised under L12 and L18 photoperiods. However, for the group that simulates the harvesting season of grapes (L6), the impact of both CG and OG intake was very similar, mainly resulting in a significant decrease of the expression of most genes in rWAT. This adipose depot was used as a representative of the visceral adipose tissue, which has been largely correlated with increased metabolic dysfunction.[37] On the other hand, animals under the L12 photoperiod increased the expression of both adipogenic and lipolytic genes in iWAT in response to CG consumption, this effect being attenuated after consuming OG. In this case, the iWAT depot represented the subcutaneous adipose tissue, which shows completely different metabolic effects than visceral WAT, contributing to maintain glucose and lipid homeostasis.[37] These results indicated that the effects of grapes on the gene expression profile of WAT were strongly dependent on the adipose depot, considering the remarkable differences between iWAT and rWAT, and suggested that grape consumption promoted a rather beneficial profile of fat storage in a photoperiod-dependent manner.

Another remarkable finding was the dramatic increase of *Ucp1* gene expression in iWAT after OG consumption during both L12 and L18. Other studies are needed to corroborate this response but considering the well-established browning effects of anthocyanins,[38–40] our results suggest a winter-like adaptation feature induced by anthocyanin consumption, which is consistent with the EE rise and upregulation of *Ucp1* in BAT in L18 and L12 groups, respectively.

Interestingly, *Lep* expression was upregulated in response to the consumption of both types of grapes in iWAT of animals adapted to L12 photoperiod. Previous results only showed a slight enhancement of Lep serum levels due to grape consumption during long summer like photoperiod,[14] but no results under L12 were reported. Also, it was shown that anthocyanin treatment enhanced Lep and Adipoq production in adipocytes,[41] which suggests that the high anthocyanin content in grapes could be responsible for this *Lep* rise. Given that animals in our study were fed chow diet, it would be interesting, in order to properly interpret these results, to reproduce these studies in a model of diet-induced obesity.

Figure 3. Effect of seasonal consumption of CG or OG on the gene expression of the key regulators of the main metabolic pathways in inguinal white adipose tissue (iWAT). Gene expression of genes related to adipogenesis (A), lipolysis (B), lipogenesis (C), browning (D), and adipokines (E) evaluated by quantitative PCR on animals fed standard chow diet + 100 mg kg^{-1} of lyophilized CG or OG for 10-week-period and submitted into short day (L6), standard (L12), or long day (L18) photoperiods. Values were normalized against vehicle group of L6. Data represent mean ± standard error of the mean (SEM) ($n = 5–6$). *Fruit*: fruit effect; *Photoperiod*: photoperiod effect; *Fruit × Photoperiod*: interaction of photoperiod and fruit treatment assessed by two-way analysis of variance (ANOVA, $p < 0.05$). Different letters denote effect of fruit on each photoperiod group determined by one-way ANOVA and Tukey's post-hoc test ($p < 0.05$).

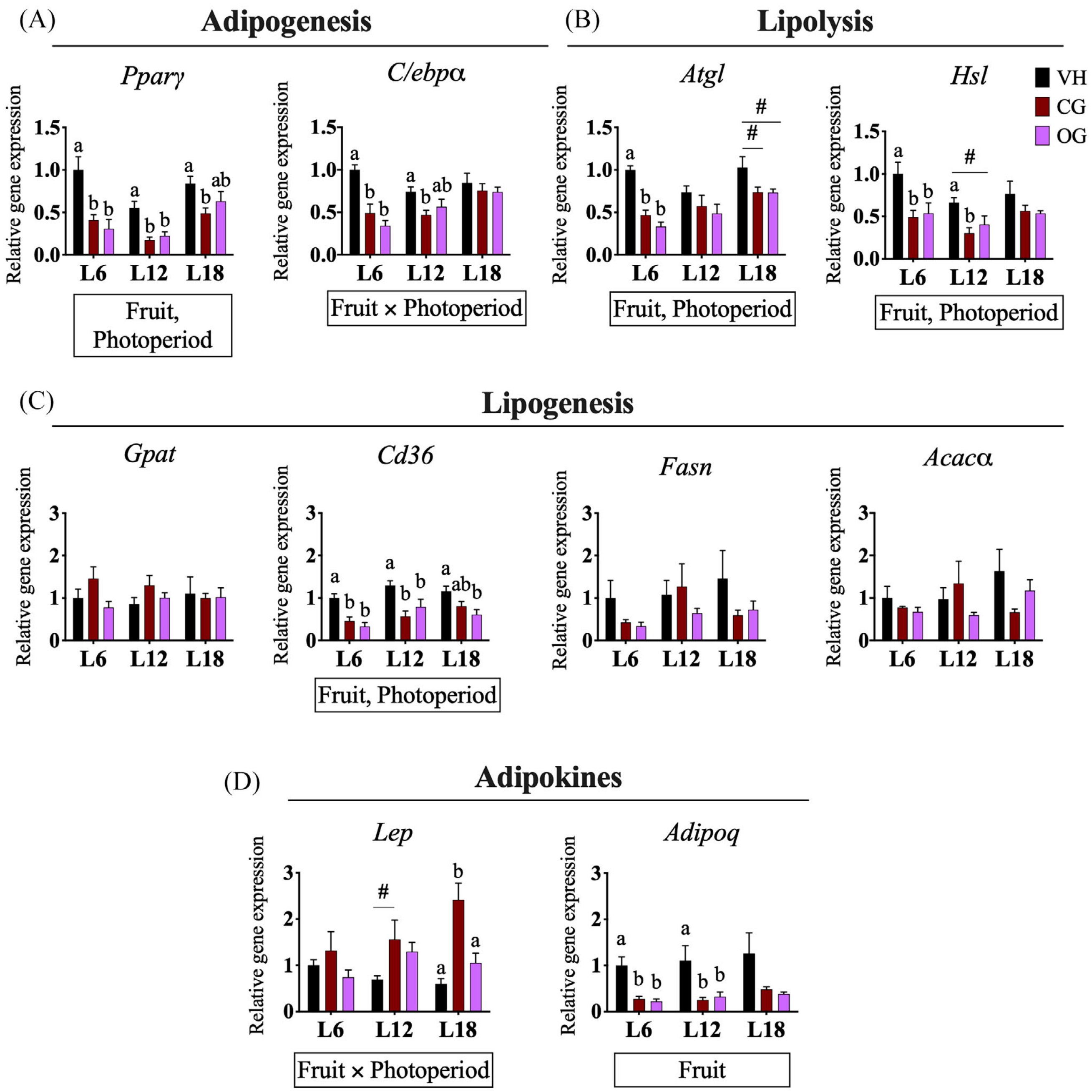


Figure 4. Effect of seasonal consumption of CG or OG on the gene expression of the key regulators of the main metabolic pathways in retroperitoneal white adipose tissue (rWAT). Gene expression of genes related to adipogenesis (A), lipolysis (B), lipogenesis (C), and adipokines (D) evaluated by quantitative PCR on animals fed standard chow diet + 100 mg kg^{-1} of lyophilized CG or OG for 10-week-period and submitted into short day (L6), standard (L12), or long day (L18) photoperiods. Values were normalized against vehicle group of L6. Data represent mean ± standard error of the mean (SEM) ($n = 5$–6). *Fruit*: fruit effect; *Photoperiod*: photoperiod effect; *Fruit × Photoperiod*: interaction of photoperiod and fruit treatment assessed by two-way analysis of variance (ANOVA) ($p < 0.05$). Different letters denote effect of fruit on each photoperiod group determined by one-way ANOVA and Tukey's post-hoc test ($p < 0.05$).

Otherwise, *Adipoq* gene expression was also affected by grape consumption in a photoperiod and adipose depot-dependent manner. On the one hand, these adipokine was upregulated in iWAT, but not in rWAT, under the short photoperiod, and only OG upregulated its expression in the subcutaneous depot when animals were exposed to the long photoperiod. However, no effects on glucose levels were detected. On the other hand, the exposure to different photoperiods did not affect *Adipoq* gene expression in rWAT, whereas consumption of both types of grapes downregulated it under all photoperiods in this visceral depot. Besides, higher glucose circulating levels were observed in response to grape consumption only in animals exposed to the L6, suggesting possible alterations on the glycemic control of these animals. In order to understand the relevance of these findings, it could be interesting to explore the effects of grapes on insulin levels and on the further utilization of glucose by the liver or the skeletal muscle. Also, to use a model of diet-induced obesity, where inflammation levels are importantly increased, could help better define the impact of grape consumption on the action of Adipoq.

In conclusion, we demonstrated a clear effect of grape consumption on EE and metabolic markers of BAT and WAT of Fischer 344 rats. This effect is photoperiod and adipose depot-dependent and probably influenced by the content of bioactive compounds in grapes, as affected by the growing conditions. Additionally, our results reinforce the role of (poly)phenols, particularly anthocyanins, as dietary cues to adapt to environmental stress such as cold temperatures and short photoperiod, as gene expression in the different adipose depots in response to out-of-season grape consumption often mimicked the pattern observed under L6. Finally, cultivation-derived differences in grape (poly)phenol composition clearly affected BAT activity. For instance, OG enhanced UCP1 in animals exposed to L12 photoperiod. However, one must consider the exploratory and preliminary nature of this study, and more evidence is needed to draw firm conclusions about the effects of seasonal grape consumption on energy and lipid metabolism. Future studies should focus on other metabolic features that describe the global metabolic situation in the organism. Also, another type of VH could be used to fully understand the particular impact of grape (poly)phenols, or of the whole fruits, in the organism.

4. Concluding Remarks

- Out-of-season grape consumption increased EE of rats, whereas in-season grape consumption reduced it.
- Grape consumption upregulated UCP1 expression in BAT of L12 animals. No effects were detected in BAT activity after grape consumption under L18, and L6 animals tended to show reduced UCP1 in response to grapes.
- Browning in iWAT was enhanced by out-of-season consumption of grapes, being significant only when consuming OG. These findings were consistent with the increased EE after grape consumption under L12 and L18.
- The expression of adipogenic and lipogenic genes in WAT was affected by grapes, depending on both the photoperiod and the adipose depot. Under L12 and L6 photoperiods, both CG and OG reduced the expression of lipid accumulation-related genes in visceral WAT. However, in subcutaneous WAT, CGs upregulated adipogenesis-related genes.
- In-season consumption of grapes downregulated the expression of Adipoq in rWAT, while glucose circulating levels were increased in these animals. Further research focused on the relevance of these effects is needed to properly interpret these results.

5. Experimental Section

Ethical Approval: All animal experimental procedures were approved by the Animal Care Institution and Ethics Committee of University Rovira i Virgili (reference number: 4249).

Fruit Characteristics and Preparation: Red Grenache grapes (*V. vinifera* L.) OG and CG were harvested at maturity in the region of Rasquera (Tarragona, Spain) and were kindly provided by a producer. According to the farmers, the antifungals copper (II) sulfate and conazole, and the pesticide chlorpyrifos were used during the conventional cultivation of the grapes. Intact grapes, including seeds, skins, and pulp, were frozen in liquid nitrogen and grinded to homogeneity. The homogenates were lyophilized in a Telstar LyoQuest lyophilizer (Thermo Fisher Scientific, Barcelona, Spain) at −85 °C.

Extraction, Quantification, and Identification of (Poly)phenols from CG and OG: The phenolic composition of grapes was extracted and characterized as described previously.[42] For (poly)phenol extraction, grape powder was extracted in a liquid–solid ratio of 80 mL g^{-1} freeze-dried grape powder with methanol 65% in water and 1% formic acid for 100 min at 72 °C under 500 rpm agitation with protection from light exposure. After the extraction, samples were centrifuged at 9500 × *g* for 10 min at 4 °C, and the supernatants were stored at −20 °C until further use. (Poly)phenol separation was achieved using a ZORBAX Eclipse XDB-C18 (150 mm × 2.1 mm i.d., 5-µm particle size) as the chromatographic column (Agilent Technologies, Palo Alto, CA, USA) equipped with a Narrow-Bore guard column (2.1 mm × 12.5 mm, 5 µm particle size). The mobile phase was water:acetic acid (95:5 v/v) (A) and acetonitrile:acetic acid (95:5 v/v) (B) in gradient mode as follows: initial conditions 0% B; 0%–30% B, 0–18 min; 30%–100% B, 18–19 min; 100% B isocratic, 19–20 min; 100%–0% B, 20–21 min. A post-run of 6 min was required for column re-equilibration. The flow rate was set at 0.5 mL min^{-1}, and the injection volume was 10 µL for all of the runs. Before the injection, all of the samples were diluted 1:1 in mobile phase A.

Animal Handling: Fifty-four ($n = 54$) male Fischer 344 rats, 8-weeks of age and with an average body weight of 186 ± 17 g, were purchased from Charles River Laboratories (Barcelona, Spain). The animals were pair-housed and randomly distributed in three different rooms according to photoperiod ($n = 18$, each). They were fed a standard chow diet (Panlab A04, Barcelona, Spain) and had access to tap water ad libitum. Photoperiod groups consisted of a L6 (6:18 h light:dark cycle), L12 (12:12 h light:dark cycle), and L18 (18:6 h light:dark cycle), emulating winter, fall/spring, and summer, respectively. Temperature was kept at 22 °C, and animals were housed in animal quarters. After an adaptation period of 4 weeks, animals under each photoperiod were weight-matched and distributed into three subgroups ($n = 6$, each): OG administration at a dose of 100 mg kg^{-1} day^{-1} water-diluted; CG administration at a dose of 100 mg kg^{-1} day^{-1} water-diluted; or VH administration, consisting of glucose (10 mg kg^{-1} day^{-1}) and fructose (10 mg kg^{-1} day^{-1}) in water (Figure S1). In all cases, lights were turned on at 9.00 a.m. and treatments were orally administered between 9.00 and 10.00 a.m. for a period of 10 weeks. Body weight and food intake were measured weekly. All animals were sacrificed by decapitation at the start of the light cycle (09:00 am) after OG, CG, or VH administration. Serum was obtained after blood clotting and centrifugation (2000 × *g*, 15 min, 4 °C). WAT and BAT were obtained and frozen immediately in liquid nitrogen.

Dosage Regimen: For CG and OG treatments, 100 mg of grape extracts per kg bw were diluted in water and daily administered to animals using a syringe. The dose was chosen based on previous studies of the group where the same values showed effects on metabolism.[12,14] VH solution consisted on 20 mg kg^{-1} bw of a glucose:fructose 1:1 solution, representing the sugars present in grapes.[7] The human equivalent dose (HED) was calculated based on body surface area,[43] and corresponded to 1.25 g day^{-1} of extract for a 70 kg adult. This dose amounts to ≈5 mg of total (poly)phenols per day, which is rapidly achieved with fruit consumption: it was estimated that the average amount of (poly)phenols consumed with fruit in Spain corresponds to 360±127 mg day^{-1}.[44]

Biometric Analysis: On the last week, animals were subjected to magnetic resonance imaging (MRI) using EchoMRI-700 (Echo Medical Systems, LLC., TX, USA) to determine body composition. Also, animals underwent 24 h of indirect calorimetry analyses using an Oxylet Pro System (Panlab). The program Metabolism 2.1.02 (Panlab) automatically calculated RQ and EE as according to the Weir equation.[45] A nitrogen excretion rate of 135 µg kg^{-1} min^{-1} was also assumed.[46] Motor activity was calculated by the number of two-leg/upright positions that occurred during 24 h.

Blood Serum Analysis: Enzymatic colorimetric kits were used for the determination of TGs (QCA, Barcelona, Spain), cholesterol, and glucose serum concentrations (WAKO, Neuss, Germany), following manufacturer's instructions. Briefly, absorbance for each sample was obtained in duplicate after the enzymatic reaction, and the concentrations for each parameter were calculated based on standard curves that were created from already known concentrations of each parameter (provided by the kits).

Gene Expression Analysis: BAT and two depots of WAT, inguinal (iWAT), and retroperitoneal (rWAT) were processed to extract total RNA using TRIzol LS Reagent (Thermo Fisher, Madrid, Spain), according to manufacturer's protocol. RNA quantity and purity were measured with a NanoDrop 1000 spectrophotometer (Thermo Scientific, Madrid, Spain). The integrity of the RNA was evaluated by RNA integrity number (RIN) through 2100 Bioanalyzer Instrument (Agilent Technologies). Reverse transcription was performed to obtain cDNA using the High-Capacity Complementary DNA Reverse Transcription Kit (Thermo Fisher). Gene expression was analyzed by quantitative PCR using the iTaq Universal SYBR Green Supermix (Bio-Rad) in the ABI prism 7900HT real-time PCR system (Applied Biosystems) using primers obtained from Biomers.net (Ulm, Germany). The forward and reverse primer sequences used in this study are presented in Supplementary Table S1. The relative expression of each gene was calculated in reference to *Ppia* housekeeping gene and normalized to the VH group of each photoperiod. The $^{\Delta\Delta}$Ct method was used and corrected for primer efficiency.[47]

Western Blot Analysis: Approximately 20 mg of BAT were homogenized in 200 µL Radio-Immunoprecipitation Assay lysis buffer (RIPA). The protein was extracted and quantified by using the BCA protein assay kit (Bio-Rad Protein Assay; BioRad, USA), following manufacturer's instructions. Protein extracts (30 µg) in Laemmli loading buffer were denatured and loaded into 10% acrylamide gels made with TGX™ Fast Cast™ Acrylamide Solutions (Bio-Rad, Barcelona, 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) with Trans-Blot Turbo Mini PVDF Transfer Packs (Bio-Rad), following the manufacturer's instructions. Efficient protein transfer was monitored by Ponceau-S stain. Next, membranes were blocked at room temperature and incubated with anti-UCP1 antibody (ab209483) (Abcam, Cambridge, UK) (diluted 1:2000), at 4 °C overnight. Thereafter, the membrane was incubated for 1 h with the secondary antibody (GE Health Care Life Sciences, Barcelona), and the protein was detected with the chemiluminescent reagent ECL Select Western Blotting Detection Reagent (GE Healthcare, Barcelona, Spain). Protein levels were quantified using ImageJ[48] and normalized to β-actin (A2228) (Sigma–Aldrich) protein levels.

Statistical Analysis: The effects of seasonal fruit supplementation in biometric, biochemical, and calorimetry parameters as well as in gene and protein expression were evaluated by both two-way ANOVA (considering the photoperiod impact) and one-way ANOVA (to assess the differences in cultivation conditions for each photoperiod) with Tukey's post hoc test. GraphPad Prism 9 (GraphPad Software, La Jolla, CA, USA) was used for all statistical analysis. The values are expressed as means ± standard error of the mean (SEM). $p < 0.05$ was considered significant.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

The authors deeply thank Dr. Niurka López and Rosa M. Pastor for their technical help and advice. This work was financially supported in part by grants from MCIN/AEI/10.13039/501100011033 (AGL2013-49500-EXP and PID2020-113739RB-I00). Nutrigenomics Research group holds the Quality Mention from the Generalitat de Catalunya (2021SGR). E.N.-M. and F.M. are recipients of a predoctoral fellowship from Universitat Rovira i Virgili-Martí Franquès (2019PMF-PIPF-73 and 2019PMF-PIPF-19, respectively) M.C.-P. is recipient of a predoctoral fellowship from Generalitat de Catalunya FI (2021 FI_B2 00150) and F.I.B. and G.A. are Serra-Húnter fellows at the Universitat Rovira i Virgili.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization, F.I.B., G.A., and B.M.; formal analysis, È.N.-M.; funding acquisition, F.I.B., B.M., and G.A.; investigation, È.N.-M., F.M., M.C.-P., and L.I.-C.; methodology, È.N.-M., F.M., and M.C.-P.; supervision, G.A.; writing – original draft, È.N.-M., G.A.; writing – review and editing, È.N.-M., X.E., E.C., Y.D., and G.A. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

biological rhythms, grapes, photoperiod, UCP1, xenohormesis

Received: February 8, 2023
Revised: May 15, 2023

[1] A. Rodriguez-Casado, *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 1097.
[2] Y. Xu, J. E. Simon, C. Welch, J. D. Wightman, M. G. Ferruzzi, L. Ho, G. M. Passinetti, Q. Wu, *J. Agric. Food Chem.* **2011**, *59*, 10586.
[3] A. Arola-Arnal, Á. Cruz-Carrión, C. Torres-Fuentes, J. Ávila-Román, G. Aragonès, M. Mulero, F. I. Bravo, B. Muguerza, L. Arola, M. Suárez, *Nutrients* **2019**, *11*, 1.
[4] J. A. Baur, D. A. Sinclair, *Am. J. Pharmacol. Toxicol.* **2008**, *6*, 356.
[5] S. V. Luca, I. Macovei, A. Bujor, A. Miron, K. Skalicka-Woźniak, A. C. Aprotosoaie, A. Trifan, *Crit. Rev. Food Sci. Nutr.* **2020**, *60*, 626.
[6] D. Carregosa, C. Pinto, M. Á. Ávila-Gálvez, P. Bastos, D. Berry, C. N. Santos, *Compr. Rev. Food Sci. Food Saf.* **2022**, 1.
[7] L. Iglesias-Carres, A. Mas-Capdevila, F. I. Bravo, G. Aragonès, A. Arola-Arnal, B. Muguerza, *Food Chem.* **2019**, *299*, 125092.
[8] L. Iglesias-Carres, A. Mas-Capdevila, F. I. Bravo, L. Arola, B. Muguerza, A. Arola-Arnal, *J. Photochem. Photobiol. B* **2019**, *199*, 111623.
[9] J. Ávila-Román, J. R. Soliz-Rueda, F. I. Bravo, G. Aragonès, M. Suárez, A. Arola-Arnal, M. Mulero, M. J. Salvadó, L. Arola, C. Torres-Fuentes, B. Muguerza, *Trends Food Sci. Technol.* **2021**, *113*, 77.
[10] M. E. Frigolet Vázquez-Vela, N. Torres, A. R. Tovar, *Arch. Med. Res.* **2008**, *39*, 715.
[11] D. X. Tan, L. C. Manchester, L. Fuentes-Broto, S. D. Paredes, R. J. Reiter, *Obes. Rev.* **2011**, *12*, 167.

[12] A. Gibert-Ramos, A. Crescenti, M. Salvadó, *Nutrients* **2018**, *10*, 1102.
[13] A. Gibert-Ramos, H. Palacios-Jordan, M. J. Salvadó, A. Crescenti, *Eur. J. Nutr.* **2020**, *59*, 621.
[14] M. Ibars, G. Aragonès, A. Ardid-Ruiz, A. Gibert-Ramos, A. Arola-Arnal, M. Suárez, C. Bladé, *Sci. Rep.* **2018**, *8*, 1.
[15] M. J. Ruiz de Azua, Á. Cruz-Carrión, B. Muguerza, A. Arola-Arnal, M. Suarez, *Nutrients* **2021**, *3643*, https://doi.org/10.3390/nu13103643
[16] A. Caimari, P. Oliver, A. Palou, *J. Nutr. Biochem.* **2012**, *23*, 1041.
[17] A. Gibert-Ramos, M. Ibars, M. Salvadó, A. Crescenti, *J. Nutr. Biochem.* **2019**, *70*, 82.
[18] B. Winkel-Shirley, *Curr. Opin. Plant Biol.* **2002**, *5*, 218.
[19] J. Turicchi, R. O'Driscoll, G. Horgan, C. Duarte, A. L. Palmeira, S. C. Larsen, B. L. Heitmann, J. Stubbs, *PLoS One* **2020**, *15*, 1.
[20] E. Yoshimura, E. Tajiri, Y. Hatamoto, S. Tanaka, *Int. J. Environ. Res. Public Health* **2020**, *17*, 1.
[21] M. Yokoya, Y. Higuchi, *PLoS One* **2019**, *14*, 1.
[22] P. M. Iannaccone, H. J. Jacob, *Dis. Model. Mech.* **2009**, *2*, 206.
[23] V. Ryu, E. Zarebidaki, E. H. Albers, B. Xue, T. J. Bartness, *Physiol. Behav.* **2018**, *190*, 11.
[24] P. D. Heideman, C. K. Bierl, M. E. Galvez, *Biol. Reprod.* **2000**, *63*, 1525.
[25] F. M. Tavolaro, L. M. Thomson, A. W. Ross, P. J. Morgan, G. Helfer, *J. Neuroendocrinol.* **2015**, *27*, 79.
[26] Y. Togo, T. Otsuka, M. Goto, M. Furuse, S. Yasuo, *Am. J. Physiol. Endocrinol. Metab.* **2012**, *303*, E777.
[27] D. Ricquier, *Front. Endocrinol. (Lausanne)* **2011**, *2*, 1.
[28] N. Osakabe, J. Hoshi, N. Kudo, M. Shibata, *Life Sci.* **2014**, *114*, 51.
[29] D. Pajuelo, S. Díaz, H. Quesada, A. Fernández-Iglesias, M. Mulero, A. Arola-Arnal, M. Salvadó, C. Bladé, L. Arola, *J. Agric. Food Chem.* **2011**, *59*, 4279.
[30] D. Pajuelo, H. Quesada, S. Díaz, A. Fernández-Iglesias, A. Arola-Arnal, C. Bladé, J. Salvadó, L. Arola, *Br. J. Nutr.* **2012**, *107*, 170.
[31] Y. Yamashita, M. Okabe, M. Natsume, H. Ashida, *Arch. Biochem. Biophys.* **2012**, *527*, 95.
[32] Y. You, X. Yuan, H. J. Lee, W. Huang, W. Jin, J. Zhan, *Food Funct.* **2015**, *6*, 401.
[33] Y. You, X. Yuan, X. Liu, C. Liang, M. Meng, Y. Huang, X. Han, J. Guo, Y. Guo, C. Ren, Q. Zhang, X. Sun, T. Ma, G. Liu, W. Jin, W. Huang, J. Zhan, *Mol. Nutr. Food Res.* **2017**, *61*, 1.
[34] P. Puigserver, B. M. Spiegelman, *Endocr. Rev.* **2003**, *24*, 78.
[35] I. Spivak, L. Fluhr, E. Elinav, *EMBO Rep.* **2022**, 1.
[36] F. F. Anhê, T. v. Varin, M. le Barz, G. Pilon, S. Dudonné, J. Trottier, P. St-Pierre, C. S. Harris, M. Lucas, M. Lemire, É. Dewailly, O. Barbier, Y. Desjardins, D. Roy, A. Marette, *Diabetologia* **2018**, *61*, 919.
[37] M. M. Ibrahim, *Obes. Rev.* **2010**, *11*, 11.
[38] V. Sandoval, A. Femenias, Ú. Martínez-Garza, H. Sanz-Lamora, J. M. Castagnini, P. Quifer-Rada, R. M. Lamuela-Raventós, P. F. Marrero, D. Haro, J. Relat, *Antioxidants* **2019**, *8*, 360.
[39] X. Han, J. Guo, Y. You, M. Yin, J. Liang, C. Ren, J. Zhan, W. Huang, *Food Funct.* **2018**, *9*, 4366.
[40] T. Xing, Y. Kang, X. Xu, B. Wang, M. Du, M. J. Zhu, *Mol. Nutr. Food Res.* **2018**, *62*, 1.
[41] T. Tsuda, Y. Ueno, H. Aoki, T. Koda, F. Horio, N. Takahashi, T. Kawada, T. Osawa, *Biochem. Biophys. Res. Commun.* **2004**, *316*, 149.
[42] L. Iglesias-Carres, A. Mas-Capdevila, L. Sancho-Pardo, F. I. Bravo, M. Mulero, B. Muguerza, A. Arola-Arnal, *Nutrients* **2018**, *1931*, https://doi.org/10.3390/nu10121931
[43] S. Reagan-Shaw, M. Nihal, N. Ahmad, *FASEB J.* **2008**, *22*, 659.
[44] A. Tresserra-Rimbau, A. Medina-Remón, J. Pérez-Jiménez, M. A. Martínez-González, M. I. Covas, D. Corella, J. Salas-Salvadó, E. Gómez-Gracia, J. Lapetra, F. Arós, M. Fiol, E. Ros, L. Serra-Majem, X. Pintó, M. A. Muñoz, G. T. Saez, V. Ruiz-Gutiérrez, J. Warnberg, R. Estruch, R. M. Lamuela-Raventós, *Nutr. Metab. Cardiovasc. Dis.* **2013**, *23*, 953.
[45] J. B. de V Weir, *J. Physiol.* **1949**, *109*, 1.
[46] F. Carraro, C. A. Stuart, W. H. Hartl, J. Rosenblatt, R. R. Wolfe, *Am. J. Physiol. Endocrinol. Metab.* **1990**, *259*, E470.
[47] M. W. Pfaffl, A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* **2001**, *29*, 45e.
[48] M. Miranda, X. Escoté, V. Ceperuelo-Mallafré, A. Megía, E. Caubet, S. Näf, J. M. Gómez, J. M. González-Clemente, V. Vicente, J. Vendrell, *Int. J. Obes.* **2010**, *34*, 679.