Time-of-day dependent effect of proanthocyanidins on adipose tissue metabolism in rats with diet-induced obesity

Marina Colom-Pellicer [1], Romina M Rodríguez [1], Èlia Navarro-Masip [1], Francisca Isabel Bravo [1], Miquel Mulero [1], Lluís Arola [1], and Gerard Aragonès [1] ✉

[1] Universitat Rovira i Virgili, Department of Biochemistry and Biotechnology, Nutrigenomics Research Group, Tarragona, Spain. ✉email: gerard.aragones@urv.cat

The authors declare no competing interests in relation to this manuscript.

ABSTRACT

BACKGROUND: Grape-seed proanthocyanidin extract (GSPE) improve white adipose tissue (WAT) expansion during diet-induced obesity. However, because adipose metabolism is synchronized by circadian rhythms, it is plausible to speculate that the bioactivity of dietary proanthocyanidins could be influenced by the time-of-day in which they are consumed. Therefore, the aim of the present study was to determine the interaction between zeitgeber time (ZT) and GSPE consumption on the functionality of WAT in rats with diet-induced obesity.

METHODS: Male Wistar rats were fed a cafeteria diet for 9 weeks. After 5 weeks, the animals were supplemented with 25 mg GSPE/kg for 4 weeks at the beginning of the light/rest phase (ZT0) or of the dark/active phase (ZT12). Body fat content was determined by nuclear magnetic resonance and histological analyses were performed in the epididymal (EWAT) and inguinal (IWAT) fat depots to determine adipocyte size and number. In addition, the expression of genes related to adipose metabolism and circadian clock function were analyzed by qPCR.

RESULTS: GSPE consumption at ZT0 was associated with a potential antidiabetic effect without affecting adiposity and energy intake and downregulating the gene expression of inflammatory markers in EWAT. In contrast, GSPE consumption at ZT12 improved adipose tissue expansion decreasing adipocyte size in IWAT. In accordance with this adipogenic activity, the expression of genes involved in fatty acid metabolism were downregulated at ZT12 in IWAT. In turn, GSPE consumption at ZT12, but not at ZT0, repressed the expression of the clock gene *Cry1* in IWAT.

CONCLUSIONS: The interaction between ZT and GSPE consumption influenced the metabolic response of WAT in a tissue-specific manner. Understanding the impact of circadian clock on adipose metabolism and how this is regulated by polyphenols will provide new insights for the management of obesity.

INTRODUCTION

The obesity epidemic is a major metabolic health problem, mainly characterized by excessive adiposity, which disrupts whole-body energy balance and body composition [1]. In mammals, white adipose tissue (WAT) is increasingly recognized as a major player in maintaining metabolic homeostasis by regulating energy metabolism [2]. In general, feeding stimulates the lipogenic pathway and storage of triglycerides in WAT, while fasting and physical activity induce lipolytic pathway activation and promote triglycerides breakdown and fatty acids release into the bloodstream to meet the needs of other tissues [3]. In addition, to properly accommodate triglycerides, WAT needs to remodel and expand itself by inducing the proliferation and differentiation of pre-adipocytes to adipocytes via adipogenesis [4]. This process is controlled by an interacting network of transcription factors, such as *peroxisome proliferator-activated receptor gamma* (PPARγ) and members of the *CCAAT/enhancer-binding proteins* (C/EBPs), operating together to coordinate the expression of many hundreds of genes responsible for establishing the phenotype of mature fat cells [5]. However, WAT expandability is not infinite and, once the limit is reached, fat is deposited in visceral adipocyte depots resulting in local and systemic metabolic alterations such as insulin resistance, dyslipidemia and chronic inflammation [6].

In this context, dietary and lifestyle interventions have been shown to be adequate to improve WAT functionality and, therefore, improve its metabolic health [7, 8]. In this sense, natural dietary polyphenols and specifically proanthocyanidins, the most structurally complex subclass of flavonoids, have exhibited, over the last few years, to play an important role in the regulation of the main metabolic transcriptional networks in WAT [9,10]. Specifically, consumption of a proanthocyanidins mixture extracted from grape seeds was shown to exert anti-hypertrophic and adipogenic activity by increasing the capacity of WAT to store and mobilize triglycerides and, consequently, inducing a healthier expansion of this tissue to match the surplus of energy provided by the cafeteria diet [11, 12].

Nevertheless, the main metabolic processes in mammals are influenced by the circadian clock, which is a complex biological timing system. This system coordinates cellular and physiological functions in periodical cycles of approximately 24 hours [13]. In fact, several studies in rodents and humans revealed that approximately 10-20% of genes in WAT have a circadian rhythm expression [14,15]. In addition, one of the most important molecular components of the mammalian circadian clock system such as *brain and muscle ARNT-like* (BMAL1), has been shown to modulate the expression of key enzymes involved in lipolysis and lipid transport in WAT, including *adipose triglyceride lipase* (ATGL), *hormone-sensitive lipase* (HSL) and *lipoprotein lipase* (LPL) [16,17]. Therefore, cellular and physiological circadian mechanisms define the moment in which metabolic processes are more active in WAT [18] and, presumably, more susceptible to be modulated [19]. In this context, it is plausible to speculate that the effectiveness of dietary polyphenols in the prevention of diet-induced obesity could depend substantially on the time-of-day in which they are consumed. Therefore, the aim of the present study was to investigate whether the functionality of WAT in response to proanthocyanidins was significantly influenced by the time-of-day of their consumption.

METHODS

Proanthocyanidins extract

The grape seed extract enriched in proanthocyanidins (GSPE) was kindly provided by *Les Dérivés Résiniques et Terpéniques* (Dax, France). According to the manufacturer, the GSPE composition used in this study contained monomers (21.3%), dimers (17.4%), trimers (16.3%), tetramers (13.3%), and oligomers (5-13 units; 31.7%) of proanthocyanidins. The phenolic composition of this extract was further analysed by Margalef et al [20].

Study design and dosage information

Thirty-two male Wistar rats weighing 250-290 g, 8 weeks of age, were purchased from *Charles*

105 *River Laboratories* (Barcelona, Spain). Animals were housed in pairs under a 12 h light-dark cycle,
106 at 22°C and fed *ad libitum* with a standard chow diet (Panlab A04, Barcelona, Spain) and tap
107 water. After one week of adaptation, animals were fed everyday *ad libitum* with cafeteria diet for
108 9 weeks. The cafeteria diet was previously described by Ribas-Latre et al [21]. Statistical power
109 analyses and sample size calculations were conducted to minimize the number of animals used
110 in the experiment. During the last 4 weeks of the cafeteria diet intervention, animals were
111 randomly divided into four groups of 8 animals each in a completely randomized design and were
112 orally supplemented at two different time points with GSPE (25 mg/kg of body weight/day) or
113 vehicle (VH). According to Zeitgeber time (ZT), two groups were supplemented at ZT0, when the
114 light turn on (VH-ZT0 and GSPE-ZT0 groups), and the other two were supplemented at ZT12, when
115 lights turned off (VH-ZT12 and GSPE-ZT12 groups). GSPE was dissolved in 450 µL of commercial
116 sweetened skim condensed milk (Nestle; 100 g: 8.9 g protein, 0.4 g fat, 60.5 g carbohydrates,
117 1175 kJ). VH groups were supplemented with the same volume of sweetened skim condensed
118 milk. Two or three days before administration, rats were trained to voluntarily lick the milk to
119 avoid oral gavage. One week prior to sacrifice, total body fat content was analysed by quantitative
120 magnetic resonance using an EchoMRI-700 (*Echo Medical Systems*, LLC., TX, USA) without
121 anaesthesia. To avoid differences related to circadian rhythms in gene expression, all animals
122 were sacrificed by decapitation 3 hours after the lights turn on (ZT3). Afterwards, blood from neck
123 was collected and centrifuged (1 500 x g, 15 min, 4°C) to obtain serum. In addition, epididymal
124 and inguinal WAT depots (EWAT and IWAT, respectively) were excised, weighted and immediately
125 frozen in liquid nitrogen. Serum and adipose depots were stored at −80°C until further use.

126 The Animal Ethics Committee of Universitat Rovira i Virgili approved all procedures (reference
127 number 9495 by Generalitat de Catalunya). All the above-mentioned experiments were
128 performed as authorized (European Directive 86/609/CEE and Royal Decree 223/1988 of the
129 Spanish Ministry of Agriculture, Fisheries and Food, Madrid, Spain). Investigators were not
130 blinded to the treatment groups during the following analyses.

Measurement of circulating biomarkers

Serum levels of insulin, leptin, adiponectin, and monocyte chemoattractant protein-1 (MCP-1) were measured using mouse/rat-specific immunometric sandwich enzyme-linked immunosorbent assay (ELISA) kit purchased from Millipore Ibérica (Madrid, Spain). Enzymatic colorimetric kits were used for the determination of serum glucose, triglycerides and total cholesterol (QCA, Barcelona, Spain) and non-esterified fatty acids (NEFA, or free fatty acids) (Wako, CA, USA). Homeostasis model assessment-estimated insulin resistance (HOMA-IR) index was calculated from insulin and glucose serum levels.

Adipose tissue histology

This study focuses on two key white adipose depots: inguinal subcutaneous WAT (IWAT) and visceral WAT derived from the epididymal (gonadal) fat pad (EWAT). IWAT and EWAT have an important role in metabolic homeostasis. In fact, in rodent models, surgical removal of IWAT can lead to metabolic dysfunction [22,23]. In contrast, transplantation of IWAT into the visceral cavity of mice leads to improved glucose homeostasis and body composition [24]. In addition, EWAT is one of the most used adipose tissues because it makes up the largest portion of the total adipose tissue of an abdominal cavity and allows more accurate weighing due to easy extraction. Small pieces of frozen IWAT and EWAT were thawed and fixed in 4% formaldehyde. Paraffin blocks, hematoxylin-eosin staining and the calculations for area, volume and number of adipocytes were performed following Gibert-Ramos et al [25]. The frequency of adipocyte size distribution across the tissue was calculated by distributing all counted cells of each sample into two groups according to their area, <3 000μm^2 or >3 000μm^2; then, the number of total counted adipocytes was used to calculate the percentage of adipocytes in both categories.

Gene expression analysis

Total RNA from IWAT and EWAT was extracted and quantified following Gibert-Ramos et al [25]. The integrity of the RNA was evaluated by RNA integrity number (RIN) through 2100 Bioanalyzer

Instrument (Agilent Technologies). cDNA was synthesized and amplified following Ibars et al [26]. The candidate genes were selected based on previous studies [12,21,25] as well as on their implication on the most important metabolic pathways of adipose tissue including adipogenesis, lipid metabolism, thermogenesis, adipokine expression, inflammation and glucose uptake. The primers used for the different genes are described in Supplementary Information Table S1 and were obtained from *Biomers.net* (Ulm, Germany). The relative expression of each gene was calculated according to *Cyclophilin peptidylprolyl isomerase A* (*Ppia*) mRNA levels and normalized to the levels measured in the corresponding control group. The ΔΔCt method was used and corrected for primer efficiency [27].

Statistical analysis

Data are expressed as the mean±SEM (n=6-8). Grubbs' test was used to detect outliers, which were discarded before subsequent analyses. Treatment (T), administration time (ZT) and treatment × administration time interaction (T×ZT) effects within groups were determined by performing two-way analysis of variance (ANOVA) followed by Tukey's post-hoc test when F was significant [2×2 factorial designs: treatment (GSPE or VH) x administration time (ZT0 or ZT12)]. Only significant F values were shown in the text as well as in Supplementary information. Statistical tests were performed using XL-Stat 2017 software (Addinsoft, Paris, France) and graphics were prepared using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). A *P* value ≤0.05 was considered statistically significant.

RESULTS

GSPE consumption at ZT0 improved insulin sensitivity without affecting adiposity and energy intake

As shown in Table 1, final body weight, body weight gain, body fat content and energy intake were not significantly affected by GSPE consumption and only animals supplemented with VH or

GSPE at ZT12 showed lower energy intake values than animals at ZT0 [ZT effect; *F* (1,27)=5.57; P=0.025]. In contrast, GSPE consumption significantly reduced insulin values [T effect; *F* (1,23)=4.74; *P*=0.039] (Table 2). In fact, HOMA-IR values were significantly improved in response to GSPE only at ZT0 [ZTxT effect; *F* (1,19)=4.51; P=0.046], indicating that GSPE consumption could restore insulin sensitivity in animals with obesity when it was consumed at the beginning of the light phase. No effects were observed in triglyceride, total cholesterol, non-esterified fatty acids (NEFA), proinflammatory monocyte chemoattractant protein-1 (MCP-1), leptin and adiponectin blood levels driven by GSPE administration (Table 2).

GSPE consumption reduced adipocyte size and increased adipocyte number in IWAT but not in EWAT.

Knowing that the functionality of the adipose tissue can determine metabolic disturbances associated with obesity, we next explored whether GSPE consumption could modulate adipocyte histology and WAT expansion in rats with obesity. There are important metabolic differences between WAT depots which depend on their localization (in or outside of the abdominal cavity). Hence, in this study, both size and number of adipocytes were studied in two fat pads: inguinal subcutaneous WAT (IWAT) and visceral WAT derived from the epididymal fat pad (EWAT). A representative histological image of IWAT and EWAT for each group of animals is shown at **Supplementary Information Fig. S1.**

When a two-way ANOVA test was conducted, a significant effect of GSPE consumption (T effect) was observed in IWAT histology in comparison with VH animals regardless of administration time (Fig. 1A). However, when Tukey's post hoc test was used, animals supplemented with GSPE at ZT12 significantly presented lower adipocyte area and lower frequency of larger adipocytes distribution (>3 000 μm^2) with respect to VH-ZT12 animals (**Fig. 1A**). Interestingly, comparisons between VH-ZT0 and VH-ZT12 did not result in any significant differences in IWAT, reinforcing the results obtained in response to GSPE consumption. In contrast, in EWAT, GSPE treatment

did not produce any significant change in adipocyte morphology in any group of animals (**Fig. 1B**).

GSPE consumption at ZT12 resulted in decreased gene expression of positive regulators of adipogenesis, fatty acid metabolism and glucose uptake in IWAT.

We next investigated the metabolic gene expression profile of several key regulators of adipocyte metabolism in IWAT (**Fig. 2 and Supplementary information Table S2**) and EWAT (**Fig. 3 and Supplementary information Table S3**). Additionally, the differential gene expression heatmap for each fat depot is also shown in **Supplementary Information Fig. S2.**

As illustrated in **Fig. 2**, the consumption of GSPE produced a different pattern of gene expression in IWAT depending on the ZT in which GSPE was consumed (ZTxT effect). In fact, GSPE consumption at ZT0 did not significantly affect the relative gene expression of these genes. In contrast, GSPE consumption at ZT12 produced a significant downregulation of the adipogenic factor *Pparγ* [ZTxT effect, F (1,23)=7.94; P=0.009] as well as of several genes involved in fatty acid metabolism including *Mgl* [ZTxT effect, F (1,22)=9.86; P=0.004], *Cpt1b* [ZTxT effect; F (1,21)=8.07; P=0.009] and *Cd36* [ZTxT effect, F (1,21)=7.41; P=0.012]. In addition, in this tissue, GSPE consumption at ZT12 also decreased the gene expression of *Adipoq* [ZTxT effect, F (1,21)=5.85; P=0.024] and the insulin-signaling effector *Glut4* [ZTxT effect, F (1,22)=11.25; P=0.002]. Interestingly, when VH-ZT0 and VH-ZT12 groups were compared, only a significant upregulation of *Glut4* gene expression was observed in animals supplemented at ZT12 with respect to ZT0.

GSPE consumption at ZT0 reduced the gene expression of inflammatory and thermogenic markers in EWAT.

In EWAT, as observed in IWAT, the consumption of GSPE also produced a different pattern of expression depending on the ZT in which GSPE was consumed. However, contrary to the results observed in IWAT, GSPE consumption at ZT0 resulted in a downregulation of genes involved in

lipid metabolism including *Gpat* [ZTxT effect, F (1,23)=20.75; P=0.0001] and *Ucp1* [ZTxT effect, F (1,24)=6.27; P=0.019] (**Fig. 3**). Interestingly, GSPE consumption at ZT0 also caused a significant decrease of the proinflammatory cytokine *Il6* [F (1,23)=5.69; P=0.025]. In contrast, at ZT12, a significant upregulation of *C/ebpa* [ZTxT effect, F (1,24)=17.04; P=0.0004], *Gpat* [ZTxT effect, F (1,23)=20.75; P=0.0001] and *Hsl* [ZTxT effect, F (1,24)=7.926; P=0.012] was detected in response to GSPE consumption, while no significant changes were observed in the expression of genes involved in lipid transport and glucose metabolism. Comparing VH-ZT0 and VH-ZT12 groups, the mRNA levels of *Gpat* and *Il6* were significantly downregulated at ZT12 with respect to ZT0, while A*dipoq* was significantly upregulated at ZT12.

GSPE consumption upregulated *Cry1* gene expression in IWAT at ZT12 but not at ZT0.

Different components of the circadian clock system have been shown to modulate the expression of key genes involved in adipogenesis, and lipid and glucose metabolism in WAT. Therefore, we further studied whether these changes in gene expression driven by GSPE consumption were associated with clock gene expression profiles in IWAT and EWAT. Therefore, we evaluated the expression pattern of *Bmal1* (clock-core gene), *Per2, Cry1* and *Rev-erb-a* (components of the negative loop of the circadian clock) in response to GSPE consumption (**Fig. 4 and Supplementary information Table S4**).

In IWAT, GSPE consumption was able to modulate the mRNA levels of *Bmal1*, *Cry1* and *Rev-erb-a* depending on the ZT in which GSPE was consumed. Specifically, GSPE consumption at ZT12 significantly upregulated the gene expression of *Cry1* [ZTxT effect, F (1,20)=5.23; P=0.033]. In EWAT, the consumption of GSPE also produced a different pattern of the gene expression of *Bmal1* [ZTxT effect; F (1,24)=7.66; P=0.010] and *Per2* [ZTxT effect; F (1,24)=4.33; P=0.048], while *Cry1* was upregulated at ZT0 regardless of GSPE administration.

DISCUSSION

The health effects of polyphenols are usually determined without considering the time-of-day of their consumption. However, it seems evident that biological rhythms should be considered in preclinical and clinical nutritional studies as many physiological and metabolic processes in mammals present circadian oscillations. In this sense, similar to other peripheral tissues studied from a circadian context, WAT is a metabolic organ with a tremendous plasticity that is directly influenced by circadian clock [14-19]. In addition, previous studies have demonstrated that a long-term consumption of GSPE induced a healthier expansion of WAT to match the surplus energy provided by the cafeteria diet by reducing adipocyte size and increasing fat cell number [11,12]. Unfortunately, to the best of our knowledge, any study has addressed the interaction between circadian WAT metabolism and GSPE consumption. In this context, our study was specifically designed to investigate whether the metabolic response of WAT to GSPE consumption was significantly influenced by the time-of-day in which it was consumed. For this purpose, GSPE was administered to rats with obesity during 4 weeks at the beginning of the light phase (ZT0) or, on the contrary, at the beginning of dark phase (ZT12). Our results provide, for the first time, evidence that the biological effect of GSPE on WAT metabolism depends on the ZT point of its consumption.

Although various studies have shown that polyphenols and polyphenolic extracts could prevent body weight gain and fat accumulation, there are scarce data about the effect of polyphenols at different ZT points on body fat content. In addition, in most of these studies performed with animals, the body-lowering effect in response to polyphenolic consumption has been mainly observed after the administration for a long time (<10 weeks) and/or using high doses of polyphenolic compounds (<200 mg/kg of body weight) [28,29]. Here, although our data did not demonstrate an effect of GSPE on body fat content, we report for the first time that the administration of GSPE during 4 weeks at dose of 25 mg/kg of body weight significantly altered the pattern of expansion of IWAT by decreasing adipocyte size only when GSPE was consumed

282 at the beginning of the dark phase (ZT12), while this effect was not observed when GSPE was
283 consumed at the beginning of the light phase (ZT0). In contrast, in EWAT, GSPE consumption did
284 not modulate adipocyte morphology in any ZT point, indicating that subcutaneous fat could be
285 more sensitive than visceral fat to GSPE. In mammals, when energy intake is higher than energy
286 expenditure, WAT can expand by generating more adipocytes (hyperplasia) or by storing more
287 fat in existing adipocytes (hypertrophy). Several studies have shown that hyperplasia protects
288 against insulin resistance and inflammation, whereas adipocyte hypertrophy causes an
289 overproduction of adipokines that decreases tissue insulin sensitivity and induces oxidative
290 stress and inflammation [30]. In our model of obesity using CAF diet-fed rats, subcutaneous
291 IWAT could act as a buffer for the daily excess of food intake, protecting against visceral fat
292 accumulation which is closely correlated with unhealthy metabolic complications such as
293 inflammation and insulin resistance [31].
294 Although GSPE consumption at ZT0 did not display significant changes in the histologic analysis
295 of WAT, the pro-inflammatory gene *IL-6* was notably downregulated in visceral EWAT when
296 GSPE was consumed at ZT0. In fact, visceral adipose tissue secretes more proinflammatory
297 factors than subcutaneous adipose tissue [32], and TNF-α and IL-6 may lead to insulin resistance
298 by increasing free fatty acid produced by adipocytes and reducing adiponectin synthesis [33]. In
299 addition, both TNF-α and IL-6 exhibit circadian rhythmicity in their gene expression, being
300 highest in the rest phase and lowest in the active phase [34]. Our results indicate that GSPE
301 consumption downregulated the gene expression of *IL-6* only when their expression levels
302 reach its maximum during the rest time (light phase) and did not exert any anti-inflammatory
303 effect at ZT12 when their gene expression levels are physiologically reduced during the active
304 phase (dark phase). Consistent with these results, acute resveratrol (RSV) administration in male
305 Wistar rats also showed opposite effects on proinflammatory and lipid peroxidation levels
306 depending on the time-of-day of its administration [35]. Although blood concentration of MCP-
307 1, another pro-inflammatory chemokine with a well-established systemic role in the regulation

of metabolism and insulin resistance in mammals [36], did not show significant changes in GSPE-treated animals, its concentrations were reduced when GSPE was administered at ZT0, reinforcing the putative anti-inflammatory and antidiabetic role of this polyphenolic extract when it is consumed during the rest phase.

Glucose and lipid metabolism also present circadian regulation in order to prepare the metabolic functions for the outcoming situations during the day. In this sense, it was reported that insulin sensitivity in mammals displays a circadian rhythm and that it reaches its maximum around noon and its minimum during rest phase [37]. It has been recently demonstrated that an acute ingestion of catechin-rich green tea reduces postprandial blood glucose concentrations when it was consumed in the evening but not in the morning in healthy young men [38]. Accordingly, our data demonstrated that GSPE consumption restores HOMA-IR values in animals with diet-induced obesity only when it was administered at ZT0 (the beginning of rest phase in Wistar rats) suggesting that GSPE could enhance insulin sensitivity only when it reaches its minimum during the rest time. Although several studies have previously demonstrated that GSPE consumption properly restore insulin levels in animals with obesity [39,40], our study is the first reporting such differential effects between ZT points and GSPE consumption. By contrast, in our experiment, GSPE consumption did not prevent the hyperlipidemia induced by the CAF diet in any ZT point. Several works have reported a decrement in the circulating levels of triglycerides and cholesterol in response to GSPE consumption, but others have found no significant effects [41]. These discrepancies may rely either on the differences among polyphenolic extracts, the length of treatment, the dose of polyphenols or the animal models used in the different studies.

Other important effects of polyphenols in adipose tissue lead to a decrease in adiposity by reducing adipogenesis and the release of adipokines such as leptin [42]. In our study, when GSPE was consumed at ZT12, a significant decrease in the gene expression of *Leptin* and the adipogenic gene *Pparγ* was observed in IWAT, reinforcing the robust metabolic correlation

between leptin and fat mass and contributing to the enhancement of leptin sensitivity. The mechanisms that control adipogenesis have been studied mainly *in vitro*, and little information is known under *in vivo* conditions. However, in a previous study of our group [12], the adipose tissue of rats supplemented with GSPE showed an increase in the expression of *Pref-1*, suggesting that GSPE supplementation could increase the number of adipocyte precursors in this tissue. However, further research is worthy to be conducted to assess the impact of GSPE in the proliferation of pre-adipocytes in order to elucidate its potential effect on adipogenesis.

In addition, GSPE consumption at ZT12 also resulted in a downregulation of all genes related to lipolysis, fatty acid oxidation (*Cpt1b*) and lipid transport, strongly suggesting that GSPE could prevent fat accumulation in this tissue through the reduction of adipocyte lipid uptake. In fact, it is well-established that body fat mass loss is mainly driven by lower rate of lipid uptake rather than by changes in lipid removal through lipolysis [43]. Our results suggest that GSPE consumption downregulated the expression of lipolytic genes in IWAT only when their expression levels reach its maximum at dark phase, compared with ZT0 when the expression of these lipolytic genes reaches the trough. Additionally, our results also reported a significant upregulation of *Cry1* gene in IWAT, but not in visceral EWAT, in response to GSPE consumption at ZT12. *Cry*-deficient mice increase lipid storage in adipose tissue suggesting that *Cry1* regulates lipid transport and lipolysis [44]. In addition, several authors have demonstrated that *Rev-erb-a* is an essential positive regulator of adipogenesis [45], reinforcing the hyperplasic effect of GSPE on WAT expansion when it was consumed at ZT12 compared to ZT0. In contrast, the mRNA expression of *Adipoq* gene and the glucose transporter gene *Glut4* were simultaneously decreased in response to GSPE consumption at ZT12 in this tissue. This effect of GSPE consumption had previously been reported by others in mesenteric WAT [40].

Finally, in visceral EWAT, a significant increase in the expression of both *Gpat* and *Hsl* genes was observed when GSPE was consumed at ZT12. This simultaneous activation of both catabolic and anabolic lipid pathways in response to GSPE consumption was previously reported in visceral fat

depots and *in vitro* adipocytes [46,47]. However, the metabolic meaning of this apparently futile cycle induced by GSPE consumption is still unclear.

However, as in this study the gene expression is only measured at one time point but not over time, it is not plausible to determine changes in the circadian expression profile of these genes. In addition, the potential circadian entrainment effect of the sweetened skim condensed milk used to help animals to consume GSPE properly for 4 weeks cannot also be discarded. Consequently, further studies are needed to assess whether the modification of the expression of clock genes in response to GSPE consumption was due to a phase shift, a period enlargement, or a modification in the amplitude.

In conclusion, our results indicated that the beneficial effects associated with the consumption of GSPE in animals with obesity are strongly influenced in a tissue-specific manner by the time-of-day in which it was consumed. Particularly, GSPE consumption at the beginning of light phase (ZT0) was associated with a potential antidiabetic and anti-inflammatory effect in EWAT, whereas its consumption at the beginning of the dark phase (ZT12) resulted in a hyperplasic expansion of IWAT. Therefore, it is plausible to speculate that the consumption of polyphenols at the most optimal time-of-day could properly potentiate the metabolic response of adipose tissue and therefore affect the whole-body energy metabolism contributing to the management of overweight and obesity.

REFERENCES

1. Conway B, Rene A. Obesity as a disease: No lightweight matter. Obes Rev. 2004;5:145-151.
2. Coelho M, Oliveira T, Fernandes R. Biochemistry of adipose tissue: an endocrine organ. Arch Med Sci. 2013;9:191-200.
3. Ducharme NA, Bickel PE. Minireview: Lipid droplets in lipogenesis and lipolysis. Endocrinology. 2008;149:942-949.
4. Kuri-Harcuch W, Velez-delValle C, Vazquez-Sandoval A, Hernández-Mosqueira C, Fernandez-

Sanchez V. A cellular perspective of adipogenesis transcriptional regulation. J Cell Physiol. 2019;234:1111-1129.

5. Madsen MS, Siersbaek R, Boergesen M, Nielsen R, Mandrup S. Peroxisome Proliferator-Activated Receptor and C/EBP Synergistically Activate Key Metabolic Adipocyte Genes by Assisted Loading. Mol Cell Biol. 2014;34:939-954.

6. Chait A, den Hartigh LJ. Adipose Tissue Distribution, Inflammation and Its Metabolic Consequences, Including Diabetes and Cardiovascular Disease. Front Cardiovasc Med. 2020;7:1-41.

7. Stanford KI, Middelbeek RJW, Goodyear LJ. Exercise effects on white adipose tissue: Beiging and metabolic adaptations. Diabetes. 2015;64:2361-2368.

8. Palou A, Picó C, Luisa Bonet M. Nutritional potential of metabolic remodelling of white adipose tissue. Curr Opin Clin Nutr Metab Care. 2013;16:650-656.

9. Peng J, Jia Y, Hu T, Du J, Wang Y, Cheng B, et al. GC-(4→8)-GCG, A Proanthocyanidin Dimer from Camellia ptilophylla, Modulates Obesity and Adipose Tissue Inflammation in High-Fat Diet Induced Obese Mice. Mol Nutr Food Res. 2019;63:1-12.

10. Ardévol A, Motilva MJ, Serra A, Blay M, Pinent M. Procyanidins target mesenteric adipose tissue in Wistar lean rats and subcutaneous adipose tissue in Zucker obese rat. Food Chem. 2013;141:160-166.

11. Pascual-Serrano A, Bladé C, Suárez M, Arola-Arnal A. Grape Seed Proanthocyanidins Improve White Adipose Tissue Expansion during Diet-Induced Obesity Development in Rats. Int J Mol Sci. 2018;19:2632.

12. Pascual-Serrano A, Arola-Arnal A, Suárez-García S, Bravo FI, Suárez M, Arola L, et al. Grape seed proanthocyanidin supplementation reduces adipocyte size and increases adipocyte number in obese rats. Int J Obes. 2017;41:1246-1255.

13. Patke A, Young MW, Axelrod S. Molecular mechanisms and physiological importance of circadian rhythms. Nat Rev Mol Cell Biol. 2020;21:67-84.

14. Ptitsyn AA, Zvonic S, Conrad SA, Scott LK, Mynatt RL, Gimble JM. Circadian clocks are resounding in peripheral tissues. PLoS Comput Biol. 2006;2:126-135.

15. Zvonic S, Ptitsyn AA, Conrad SA, Scott LK, Floyd ZE, Kilroy G, et al. Characterization of Peripheral

Circadian Clocks in Adipose Tissues. Diabetes. 2006;55:962-70.

16. Shostak A, Meyer-Kovac J, Oster H. Circadian Regulation of Lipid Mobilization in White Adipose Tissues. Diabetes. 2013;62:2195-203.

17. Stenvers DJ, Scheer FAJL, Schrauwen P, la Fleur SE, Kalsbeek A. Circadian clocks and insulin resistance. Nat Rev Endocrinol. 2019;15:75-89.

18. Ribas-latre A, Santos RB, Fekry B, Tamim YM, Shivshankar S, Mohamed AMT, et al. Cellular and physiological circadian mechanisms drive diurnal cell proliferation and expansion of white adipose tissue. Nat Commun. 2021;12:3482.

19. Dollet L, Zierath JR. Interplay between diet, exercise and the molecular circadian clock in orchestrating metabolic adaptations of adipose tissue. J Physiol. 2019;597:1439-1450.

20. Margalef M, Pons Z, Iglesias-Carres L, Bravo FI, Muguerza B, Arola-Arnal A. Lack of tissue accumulation of grape seed flavanols after daily long-term administration in healthy and cafeteria-diet obese rats. J Agric Food Chem. 2015;63:9996-10003.

21. Ribas-Latre A, Baselga-Escudero L, Casanova E, Arola-Arnal A, Salvadó MJ, Arola L, et al. Chronic consumption of dietary proanthocyanidins modulates peripheral clocks in healthy and obese rats. J Nutr Biochem. 2015;26:112-119.

22. Foster MT, Softic S, Caldwell J, Kohli R, DeKloet AD, Seeley RJ. Subcutaneous adipose tissue transplantation in diet-induced obese mice attenuates metabolic dysregulation while removal exacerbates it. Physiol Rep. 2013;1:1-12.

23. Cox-York K, Wei Y, Wang D, Pagliassotti MJ, Foster MT. Lower body adipose tissue removal decreases glucose tolerance and insulin sensitivity in mice with exposure to high fat diet. Adipocyte. 2015;4:32-43.

24. Tran TT, Yamamoto Y, Gesta S, Kahn CR. Beneficial Effects of Subcutaneous Fat Transplantation on Metabolism. Cell Metab. 2008;7:410-420.

25. Gibert-Ramos A, Palacios-Jordan H, Salvadó MJ, Crescenti A. Consumption of out-of-season orange modulates fat accumulation, morphology and gene expression in the adipose tissue of Fischer 344 rats. Eur J Nutr. 2020;59:621-631.

26. Ibars M, Aragonès G, Ardid-Ruiz A, Gibert-Ramos A, Arola-Arnal A, Suárez M, et al. Seasonal consumption of polyphenol-rich fruits affects the hypothalamic leptin signaling system in a

photoperiod-dependent mode. Sci Rep. 2018;8:13572.

27. Pfaffl MW. Relative quantification. Real-time PCR. 2007:64-82.

28. Pons Z, Margalef M, Bravo FI, Arola-Arnal A, Muguerza B. Chronic administration of grape-seed polyphenols attenuates the development of hypertension and improves other cardiometabolic risk factors associated with the metabolic syndrome in cafeteria diet-fed rats. Br J Nutr. 2017;117:200-208.

29. Serrano J, Casanova-Martí À, Gual A, Pérez-Vendrell AM, Blay MT, Terra X, et al. A specific dose of grape seed-derived proanthocyanidins to inhibit body weight gain limits food intake and increases energy expenditure in rats. Eur J Nutr. 2017;56:1629-1636.

30. Longo M, Zatterale F, Naderi J, Parrillo L, Formisano P, Raciti GA, et al. Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic Complications. Int J Mol Sci. 2019;20:2358.

31. Manolopoulos KN, Karpe F, Frayn KN. Gluteofemoral body fat as a determinant of metabolic health. Int J Obes. 2010;34:949-959.

32. Hamdy O, Porramatikul S, Al-Ozairi E. Metabolic Obesity: The Paradox Between Visceral and Subcutaneous Fat. Curr Diabetes Rev. 2006;2:367-373.

33. Medina EA, Afsari RR, Ravid T, Sianna Castillo S, Erickson KL, Goldkorn T. Tumor necrosis factor-α decreases Akt protein levels in 3T3-L1 adipocytes via the caspase-dependent ubiquitination of Akt. Endocrinology. 2005;146:2726-2735.

34. Cermakian N, Lange T, Golombek D, Sarkar D, Nakao A, Shibata S, et al. Crosstalk between the circadian clock circuitry and the immune system. Chronobiol Int. 2013;30:870-888.

35. Gadacha W, Ben-attia M, Bonnefont-Rousselot D, Aouani E, Chanem-Boughanmi N, Touitou Y. Resveratrol opposite effects on rat tissue lipoperoxidation: pro-oxidant during day-time and antioxidant at night. Redox Rep. 2009;14:154-8.

36. Rull A, Camps J, Alonso-villaverde C, Joven J. Insulin Resistance, Inflammation, and Obesity: Role of Monocyte Chemoattractant Protein-1 (or CCL2) in the Regulation of Metabolism. Mediators Inflamm. 2010;2010:326580.

37. Carrasco-benso MP, Rivero-gutierrez B, Lopez-Minguez J, Anzola A, Diez-Noguera A, Madrid JA, et al. Human adipose tissue expresses intrinsic circadian rhythm in insulin sensitivity. FASEB J. 1026;20:3117-3123.

38. Takahashi M, Ozaki M, Miyashita M, Fukazawa M, Nakaoka T, Wakisaka T, et al. Effects of timing of acute catechin-rich green tea ingestion on postprandial glucose metabolism in healthy men. J Nutr Biochem. 2019;73:108221.
39. Montagut G, Onnockx S, Vaqué M, Bladé C, Blay M, Fernández-Larrea J, et al. Oligomers of grape-seed procyanidin extract activate the insulin receptor and key targets of the insulin signaling pathway differently from insulin. J Nutr Biochem. 2010;21:476-481.
40. Montagut G, Bladé C, Blay M, Fernández-Larea J, Pujadas G, Salvadó MJ, et al. Effects of a grapeseed procyanidin extract (GSPE) on insulin resistance. J Nutr Biochem. 2010;21:961-967.
41. Arola L, Salvado M. Hypolipidemic effects of proanthocyanidins and their underlying biochemical and molecular mechanisms. Mol Nutr Food Res. 2010:54:37-59.
42. Sandoval V, Sanz-Lamora H, Arias G, Marrero PF, Haro D, Relat J. Metabolic impact of flavonoids consumption in obesity: From central to peripheral. Nutrients. 2020;12:1-55.
43. Arner P, Bernard S, Appelsved L, Fu KY, Andersson DP, Salehpour M, et al. Adipose lipid turnover and long-term changes in body weight. Nat Med. 2019;25:1385-1389.
44. Barclay JL, Shostak A, Leliavski A, Tsang AH, Jöhren O, Müller-Fielitz H, et al. High-fat diet-induced hyperinsulinemia and tissue-specific insulin resistance in Cry-deficient mice. Am J Physiol Endocrinol Metab. 2013;304:1053-1063.
45. Kumar N, Solt LA, Wang Y, Rogers PM, Bhattacharyya G, Kamenecha TM, et al. Regulation of adipogenesis by natural and synthetic REV-ERB ligands. Endocrinology. 2010;151:3015-3025.
46. Caimari A, Del Bas JM, Crescenti A, Arola L. Low doses of grape seed procyanidins reduce adiposity and improve the plasma lipid profile in hamsters. Int J Obes. 2013;37:576-583.
47. Pinent M, Bladé MC, Salvadó MJ, Arola L, Ardévol A. Intracellular mediators of procyanidin-induced lipolysis in 3T3-L1 adipocytes. J Agric Food Chem. 2005;53:262-266.

DATA AVAILABILITY

Application for datasets generated during and/or analyzed during the current study may be considered by the corresponding author on reasonable request.

ACKNOWLEDGEMENTS

We gratefully acknowledge the aid of laboratory technicians Niurka Dariela Llópiz and Rosa Pastor from Universitat Rovira i Virgili.

FUNDING

This research was supported by a grant (AGL2013-40707-R and AGL2016-77105-R) from the Spanish government. MC-P received a pre-doctoral fellowship from the government of Catalonia (2021 FI_B2 00105). RMR and EN-M received a grant for PhD students from the Universitat Rovira i Virgili (2018PMF-PIPF-11 and 2019PMF-PIPF-73, respectively). GA, MM and FIB are Serra-Húnter fellows at the Universitat Rovira i Virgili.

AUTHOR CONTRIBUTIONS

MC-P, MM, FIB, LA, and GA designed the research; MC-P and RMR performed the experiments and collected the data; MC-P, MM, FIB, LA and GA analyzed the data and interpreted the results; MC-P, EN-M and GA wrote the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare no competing interests in relation to this manuscript.

ADDITIONAL INFORMATION

Supplementary information is available on International Journal of Obesity's website.

Correspondence and requests for materials should be addressed to Gerard Aragonès.

Table and Figure Legends

Fig. 1 Adipocyte histology of IWAT (a) and EWAT (b). Animals were fed with a cafeteria diet for 9 weeks and treated with GSPE (25 mg/kg of body weight) or vehicle (VH) the last 4 weeks of the study when the lights turned on (ZT0) or when the lights turned off (ZT12). For adipocyte area distribution, adipocytes were divided in two groups according to their areas (<3000 or >3000 µm2). Results are presented as the mean ± SEM. (n=6). T, treatment effect using two-way ANOVA ($P < 0.05$) and Tukey's multiple comparisons post hoc test (a, b).

Fig. 2 Effects of GSPE on the expression of genes related to adipose metabolism in IWAT. Animals were fed with a cafeteria diet for 9 weeks and treated with GSPE (25 mg/kg of body weight) or vehicle (VH) the last 4 weeks of the study when the lights turned on (ZT0) or when the lights turned off (ZT12). The gene expression was measured by qPCR and normalized by *Ppia* gene expression. The relative expression (presented as fold-change) was normalized to VH-ZT0 group. Results are presented as the mean ± SEM (n=6-8). T, treatment effect; ZTxT, interaction of the supplementation time and treatment effect using two-way ANOVA ($P < 0.05$) and Tukey's multiple comparisons post hoc test (a, b). *Pparg*, peroxisome proliferator-activated receptor γ; *C/ebp α*, CCAAT/enhancer-binding protein alpha; *Acaca*, acetyl-CoA carboxylase alpha; *Fasn*, fatty acid synthase; *Gpat*, glycerol-3-phosphate acyltransferase; *Atgl*, adipose triglyceride lipase; *Hsl*, hormone-sensitive lipase; *Mgl*, monoacylglycerol lipase; *Cpt1b*, carnitine palmitoyltransferase 1B; *Lpl*, lipoprotein Lipase; *Cd36*, cluster of differentiation 36; *Fabp4*, fatty Acid Binding Protein 4; *Lep*, leptin; *Obrb*, leptin receptor; *Adipoq*, adiponectin; *Ucp1*, uncoupling protein 1; *Prdm16*, PR domain containing 16; *Tnfα*, tumor necrosis factor alpha; *Il6*, interleukin 6; *G6pd*, glucose-6-phosphate dehydrogenase; *Glut4*, glucose transporter 4; *Irs2*, insulin receptor substrate 2.

Fig. 3 Effects of GSPE on the expression of genes related to adipose metabolism in EWAT. Animals were fed with a cafeteria diet for 9 weeks and treated with GSPE (25 mg/kg of body

548 weight) or vehicle (VH) the last 4 weeks of the study when the lights turned on (ZT0) or when
549 the lights turned off (ZT12). The gene expression was measured by qPCR and normalized by
550 *Ppia* gene expression. The relative expression (presented as fold-change) was normalized to VH-
551 ZT0 group. Results are presented as the mean ± SEM (n=6-8). ZT, effect of supplementation
552 time; T, treatment effect; ZTxT, interaction of the supplementation time and treatment effect
553 using two-way ANOVA ($P < 0.05$) and Tukey's multiple comparisons post hoc test (a, b). Pparg,
554 peroxisome proliferator-activated receptor γ; C/ebp α, CCAAT/enhancer-binding protein alpha;
555 Acaca, acetyl-CoA carboxylase alpha; Fasn, fatty acid synthase; Gpat, glycerol-3-phosphate
556 acyltransferase; Atgl, adipose triglyceride lipase; Hsl, hormone-sensitive lipase; Mgl,
557 monoacylglycerol lipase; Cpt1b, carnitine palmitoyltransferase 1B; Lpl, lipoprotein Lipase; Cd36,
558 cluster of differentiation 36; Fabp4, fatty Acid Binding Protein 4; Lep, leptin; Obrb, leptin
559 receptor; Adipoq, adiponectin; Ucp1, uncoupling protein 1; Prdm16, PR domain containing 16;
560 Tnfα, tumor necrosis factor alpha; Il6, interleukin 6; G6pd, glucose-6-phosphate dehydrogenase;
561 Glut4, glucose transporter 4; Irs2, insulin receptor substrate 2.

562 **Fig. 4 Effects of GSPE on the expression of clock genes in IWAT (a) and EWAT (b).** Animals were
563 fed with a cafeteria diet for 9 weeks and treated with GSPE (25 mg/kg of body weight) or vehicle
564 (VH) the last 4 weeks of the study when the lights turned on (ZT0) or when the lights turned off
565 (ZT12). The gene expression was measured by qPCR and normalized by *Ppia* gene expression.
566 The relative expression (presented as fold-change) was normalized to VH-ZT0 group. Results are
567 presented as the mean ± SEM (n=6-8). ZT, effect of supplementation time; T, treatment effect;
568 ZTxT, interaction of the supplementation time and treatment effect; ns, no significant
569 differences using two-way ANOVA ($P < 0.05$) and Tukey's multiple comparisons post hoc test (a,
570 b). Abbreviations: *Bmal1*, brain and muscle ARNT-Like 1 (aryl hydrocarbon receptor nuclear
571 translocator-like); *Cry1*, cryptochrome circadian regulator 1; *Per2*, period circadian regulator 2;
572 *Rev-erb-α*, nuclear receptor subfamily 1, group D, member 1 (Nr1d1).

Table 1. Body weight and adiposity in rats fed a CAF diet and supplemented with GSPE or vehicle (VH) when the lights turn on (ZT0) or when then the lights turn off (ZT12).

	VH-ZT0	GSPE-ZT0	VH-ZT12	GSPE-ZT12	Two-way ANOVA
Final body weight (g)	484.88 ± 9.3	483.69 ± 10.0	483.81 ± 21.4	485.21 ± 12.1	ns
Body weight gain [1] (g)	55.26 ± 2.6	54.50 ± 5.2	54.56 ± 4.0	52.64 ± 4.5	ns
Body fat content [2] (%)	17.78 ± 1.3	18.39 ± 1.3	21.93 ± 0.7	18.34 ± 1.2	ns
IWAT (%)	2.20 ± 0.2	2.19 ± 0.2	2.40 ± 0.3	2.11 ± 0.2	ns
EWAT (%)	3.53 ± 0.2	3.73 ± 0.4	3.85 ± 0.5	3.62 ± 0.3	ns
Energy intake [3] (kJ/day)	2099.98 ± 172.8	1842.42 ± 72.8	1746.69 ± 85.1	1666.58 ± 71.4	ZT

[1] Body weight gain was calculated as the difference between body weight measured at the beginning and at the end of the treatment period (from week 6 to week 9). [2] Body fat content was assessed by quantitative magnetic resonance one week prior to sacrifice. [3] Accumulated food intake was calculated as the difference between food intake at week 9 and food intake at week 6. ns, no significant differences; ZT, effect of supplementation time using two-way ANOVA ($P < 0.05$).CAF, cafeteria; EWAT, epididymal white adipose tissue; GSPE, grape-seed proanthocyanidins extract; IWAT, inguinal white adipose tissue; VH, vehicle.

Table 2. Serum metabolic variables in rats fed a CAF diet and supplemented with GSPE or vehicle (VH*)* when the lights turn on (ZT0) or when then the lights turn off (ZT12).

	VH-ZT0	GSPE-ZT0	VH-ZT12	GSPE-ZT12	Two-way ANOVA
Glucose (mM)	8.16 ± 0.3 ab	8.49 ± 0.2 a	7.32 ± 0.2 b	8.05 ± 0.4 ab	ZT
Insulin (ng/mL)	8.60 ± 1.0	5.53 ± 0.9	7.44 ± 0.6	6.51 ± 0.6	T
HOMA-IR	76.13 ± 11.8 [a]	38.93 ± 9.7 [b]	54.55 ± 4.9 [ab]	57.01 ± 7.5 [ab]	ZT x T
Triglycerides (mg/dL)	200.2 ± 10.5	182.9 ± 18.8	187.6 ± 8.8	218.4 ± 26.1	ns
Cholesterol (mg/dL)	150.6 ± 9.2	152.4 ± 6.4	138.7 ± 3.8	153.7 ± 12.2	ns
NEFA (mg/dL)	31.78 ± 4.3	30.48 ± 2.5	22.95 ± 2.6	25.85 ± 2.4	ZT
MCP-1 (pg/mL)	343.7 ± 33.4	287.1 ± 24.0	375.3 ± 20.4	381.3 ± 25.0	ns
Adiponectin (µg/mL)	26.89 ± 2.4	25.06 ± 1.9	29.49 ± 2.8	26.48 ± 2.4	ns
Leptin (ng/mL)	35.81 ± 4.7	40.62 ± 2.8	35.86 ± 3.4	33.79 ± 3.6	ns

ZT, effect of supplementation time; T, effect of treatment; ZTxT, interaction of the supplementation time and treatment; ns, no significant differences using two-way ANOVA ($P < 0.05$) and Tukey's multiple comparisons post hoc test (a, b). CAF, cafeteria; GSPE, grape-seed proanthocyanidins extract; HOMA-IR, homeostatic model assessment-insulin resistance; MCP-1, Monocyte chemoattractant protein-1; NEFA, non- esterified fatty acids; VH, vehicle;

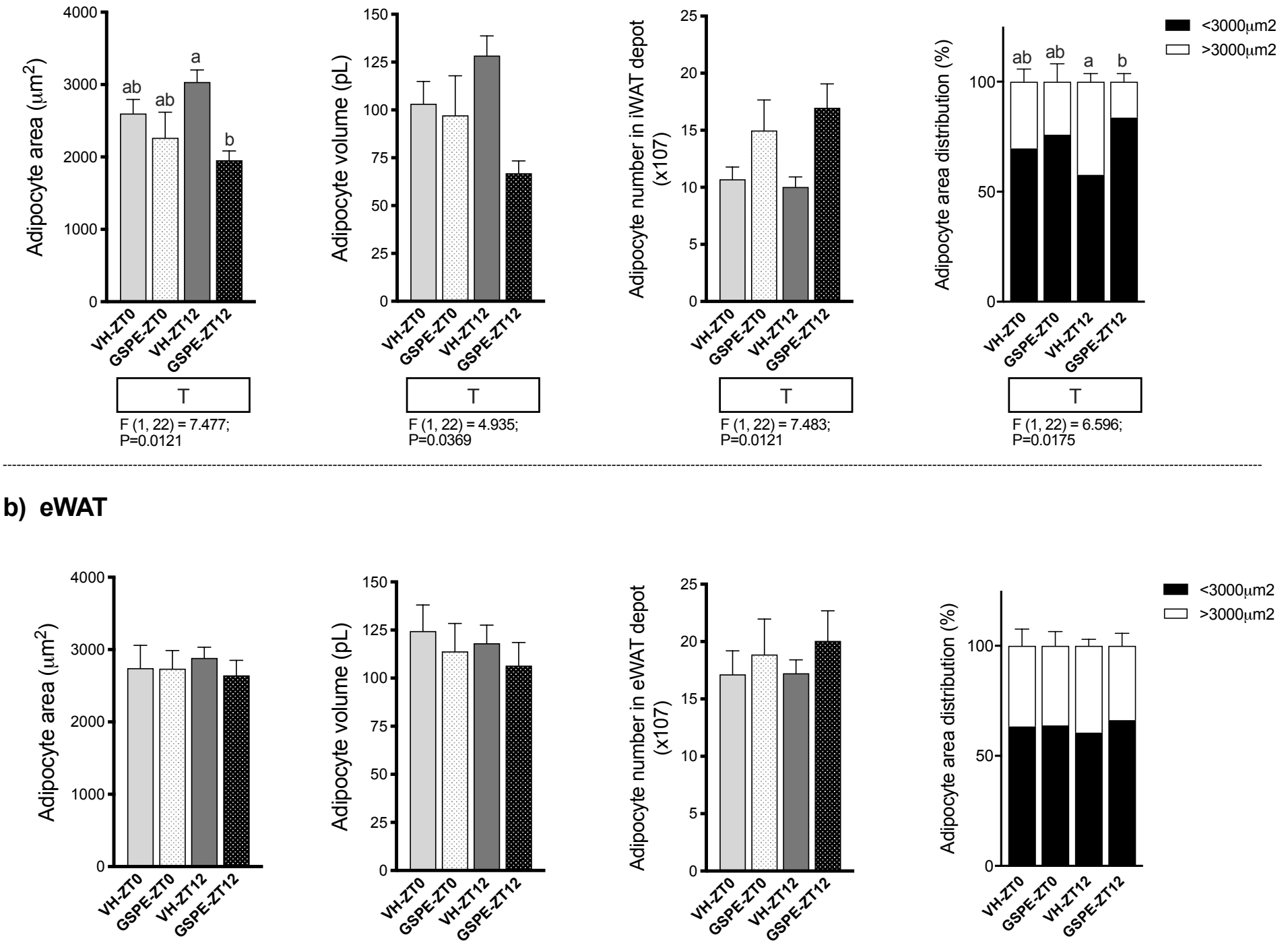
a) iWAT
Adipocyte area (μm2)
4000
3000
2000
1000
0
ab
ab
a
b
VH-ZT0
GSPE-ZT0
VH-ZT12
GSPE-ZT12
T
F (1, 22) = 7.477;
P=0.0121
Adipocyte volume (pL)
150
125
100
75
50
25
0
T
F (1, 22) = 4.935;
P=0.0369
Adipocyte number in iWAT depot (x107)
25
20
15
10
5
0
T
F (1, 22) = 7.483;
P=0.0121
Adipocyte area distribution (%)
100
50
0
ab
ab
a
b
<3000μm2
>3000μm2
T
F (1, 22) = 6.596;
P=0.0175
b) eWAT
Adipocyte area (μm2)
4000
3000
2000
1000
0
Adipocyte volume (pL)
150
125
100
75
50
25
0
Adipocyte number in eWAT depot (x107)
25
20
15
10
5
0
Adipocyte area distribution (%)
100
50
0
<3000μm2
>3000μm2

Figure 1

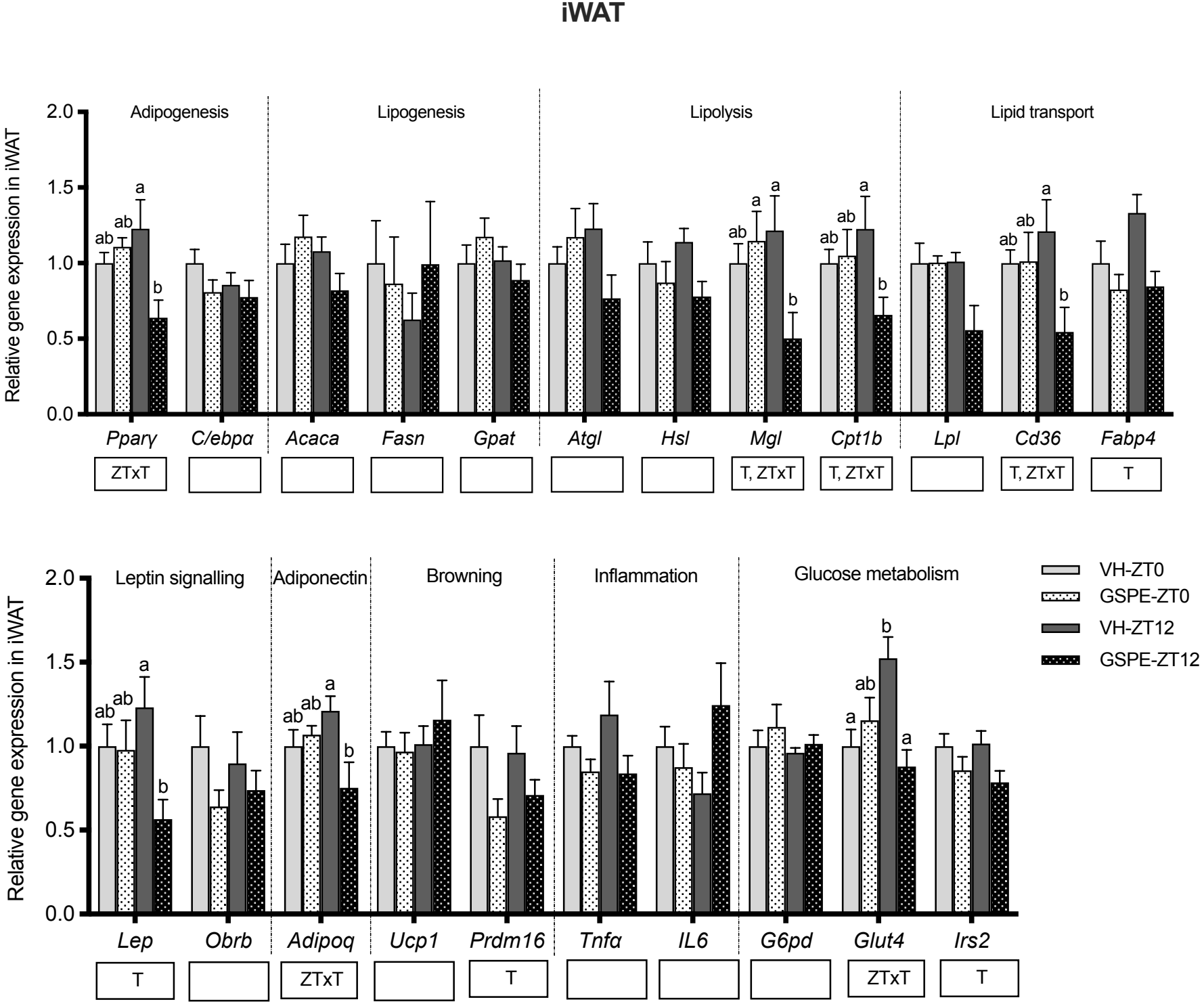
iWAT
Relative gene expression in iWAT
2.0
1.5
1.0
0.5
0.0
Adipogenesis
Lipogenesis
Lipolysis
Lipid transport
Pparγ
C/ebpα
Acaca
Fasn
Gpat
Atgl
Hsl
Mgl
Cpt1b
Lpl
Cd36
Fabp4
ZTxT
T, ZTxT
T
Leptin signalling
Adiponectin
Browning
Inflammation
Glucose metabolism
Lep
Obrb
Adipoq
Ucp1
Prdm16
Tnfα
IL6
G6pd
Glut4
Irs2
VH-ZT0
GSPE-ZT0
VH-ZT12
GSPE-ZT12

Figure 2

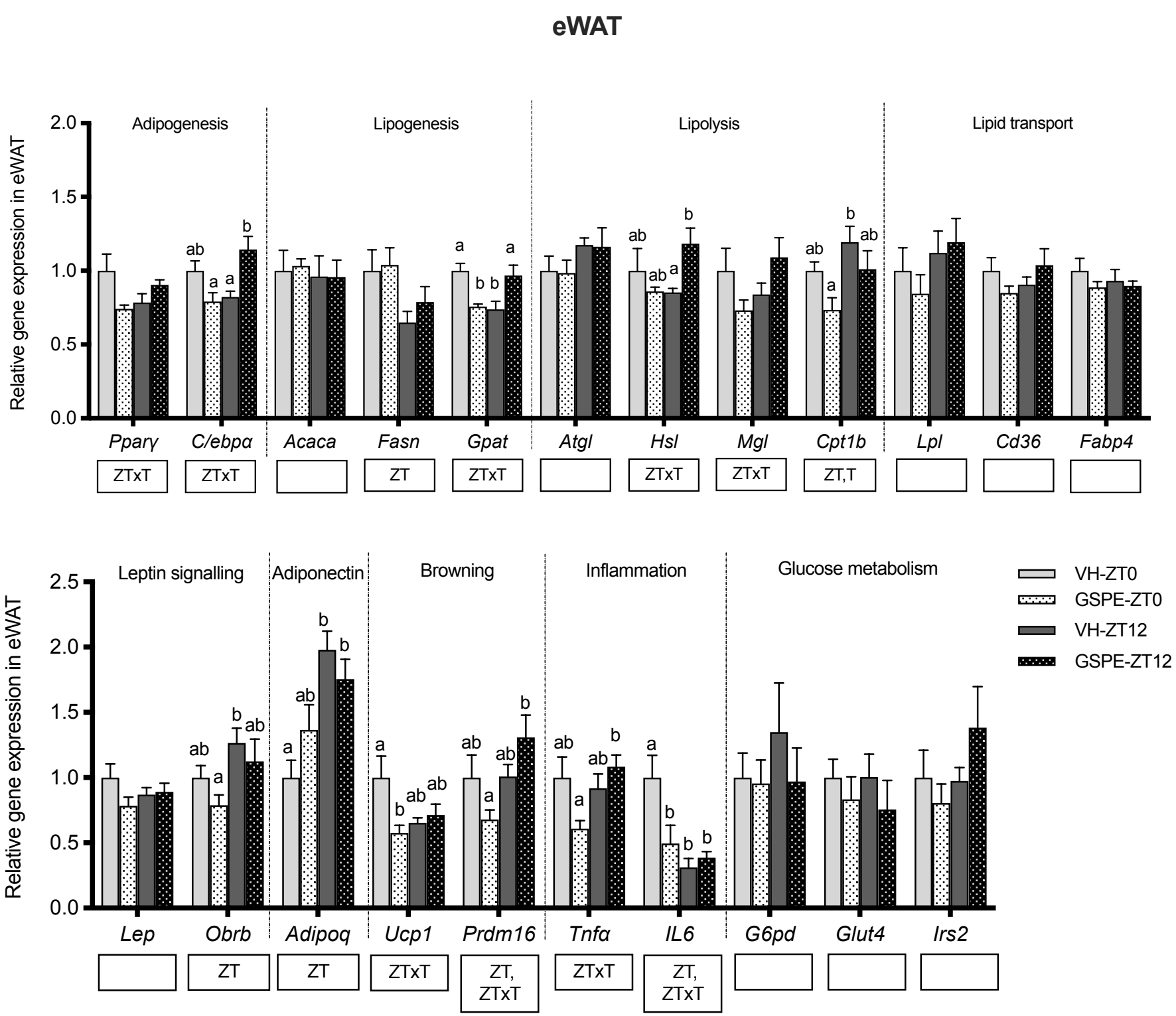
eWAT
Relative gene expression in eWAT
Adipogenesis
Lipogenesis
Lipolysis
Lipid transport
Pparγ
C/ebpα
Acaca
Fasn
Gpat
Atgl
Hsl
Mgl
Cpt1b
Lpl
Cd36
Fabp4
ZTxT
ZTxT
ZT
ZTxT
ZTxT
ZTxT
ZT,T
Leptin signalling
Adiponectin
Browning
Inflammation
Glucose metabolism
VH-ZT0
GSPE-ZT0
VH-ZT12
GSPE-ZT12
Lep
Obrb
Adipoq
Ucp1
Prdm16
Tnfα
IL6
G6pd
Glut4
Irs2
ZT
ZT
ZTxT
ZT, ZTxT
ZTxT
ZT, ZTxT

Figure 3

Clock genes

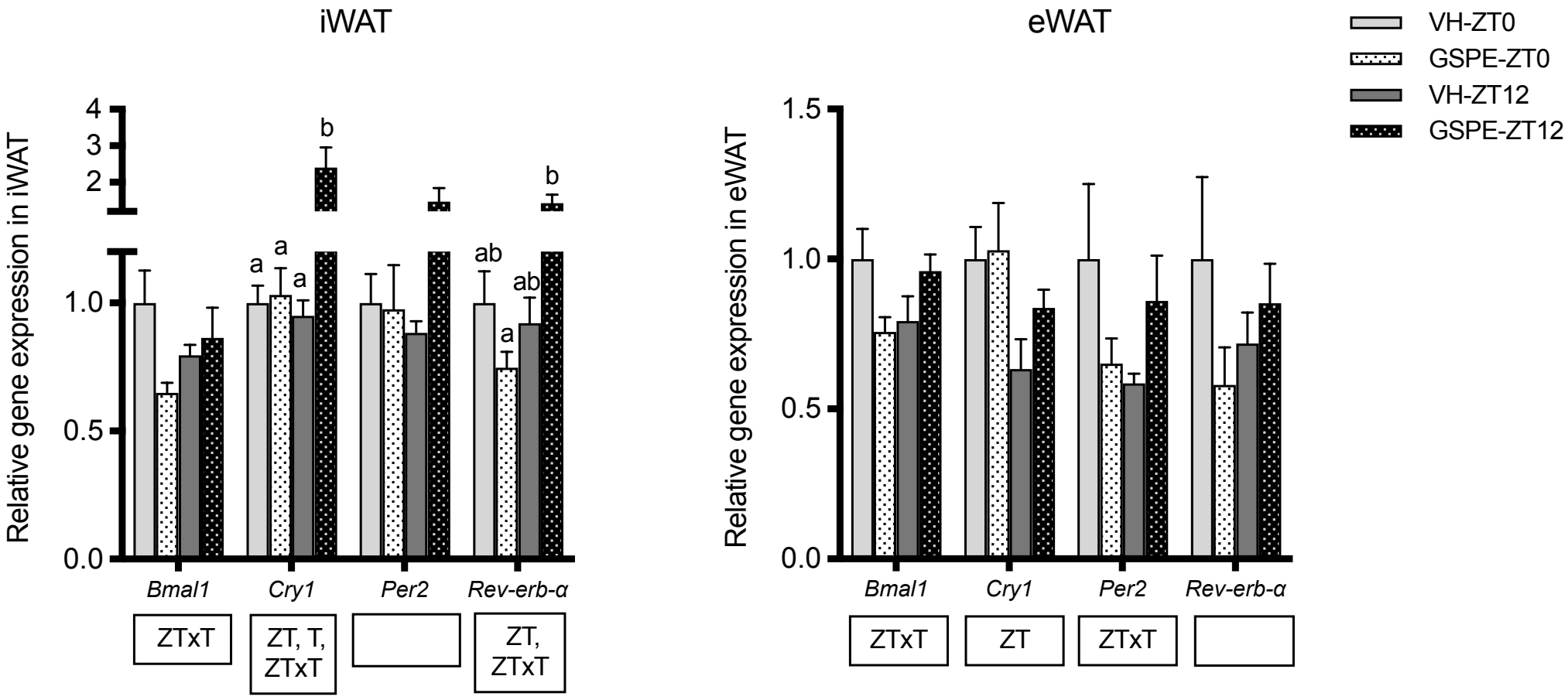

Figure 4