The Central Clock Neurons Regulate Lipid Storage in Drosophila 

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Abstract

A proper balance of lipid breakdown and synthesis is essential for achieving energy homeostasis as alterations in either of these processes can lead to pathological states such as obesity. The regulation of lipid metabolism is quite complex with multiple signals integrated to control overall triglyceride levels in metabolic tissues. Based upon studies demonstrating effects of the circadian clock on metabolism, we sought to determine if the central clock cells in the Drosophila brain contribute to lipid levels in the fat body, the main nutrient storage organ of the fly. Here, we show that altering the function of the Drosophila central clock neurons leads to an increase in fat body triglycerides. We also show that although triglyceride levels are not affected by age, they are increased by expression of the amyloid-beta protein in central clock neurons. The effect on lipid storage seems to be independent of circadian clock output as changes in triglycerides are not always observed in genetic manipulations that result in altered locomotor rhythms. These data demonstrate that the activity of the central clock neurons is necessary for proper lipid storage.

Introduction

Throughout evolution, the ability of humans to convert glucose to triglyceride for long-term storage has provided a competitive advantage during times of famine. However, in our current Western society where food is abundantly available, this thrifty phenotype has resulted in excess fat accumulation leading to 65% of adults in the United States being overweight and 30% being obese [1]. Clearly, a proper balance of the synthesis and breakdown of lipids is essential for reaching metabolic homeostasis, but the mechanisms responsible for controlling these processes are still not fully understood.

The regulation of lipid metabolism is a very complex process, utilizing a number of signals and pathways leading to lipid synthesis, breakdown or both [2]. Recent research has focused on understanding the regulation of lipid metabolism in liver and adipose tissue by the brain (reviewed in [3,4]). In mammals, the arcuate nucleus (ARC) of the hypothalamus serves as a main regulator of energy homeostasis by integrating signals from many circulating hormones. The ARC also receives neural inputs from other regions of the hypothalamus, one of these being the suprachiasmatic nucleus (SCN), the site of the central circadian clock [5]. The circadian system is, in fact, known to be a major regulator of metabolic activity, with profound metabolic phenotypes reported in clock mutant animals [6,7]. However, analysis of underlying mechanisms has focused on autonomous effects of clocks located in metabolic tissues such as the control of gene expression by such clocks as well as interactions between clock proteins and metabolic factors in these tissues [8,9,10]. Despite the connection between the ARC and the SCN, little is known about the contribution of the central clock to metabolic processes.

The fruit fly, Drosophila melanogaster, is a well-established model of circadian rhythms and has recently become a powerful model to study the regulation of metabolism [11]. In Drosophila, as in mammals, the central clock is found in specific neurons of the brain, but clocks also exist in other body tissues [12,13,14]. However, effects of these different clocks on metabolic activity are poorly understood. We showed recently that the Drosophila fat body (equivalent of mammalian liver and adipose tissue) contains a circadian clock, which regulates the storage of glycogen and triglycerides [15]. Clocks in neurons also affect glycogen storage, but the specific neurons responsible were not identified and the control of triglyceride levels by neuronal clocks was not assessed [15]. Here, we sought to explore a role of the central clock neurons in the accumulation of lipids. We report that knocking down the function of the circadian gene, Clock (Ckr) in central clock cells leads to increased triglycerides in the fly’s fat body. We observe a similar phenotype when we trigger premature degeneration in these neurons. However, triglyceride levels are normal in arrhythmic flies that express the heat-sensitive ion channel dTRPA1 in the PDF neurons and in Pdf⁴⁷ mutants, suggesting that these neurons control fat storage independently of the circadian rest/activity output. In addition, over-expression of the clock gene, timeless (tim), in these neurons does not affect triglycerides although it reduces behavioral rhythmicity. Together, these findings indicate a non-circadian
role for the central clock neurons in controlling overall energy homeostasis.

**Materials and Methods**

**Fly genetics**

Flies were grown on standard cornmeal molasses medium at room temperature. Prior to each experiment, 0–3 day old females were entrained for 2–3 days in a 12 h:12 h light:dark cycle at 25°C. For dTRPA1 experiments, flies were reared at 18–21°C and 0–3 day old flies were shifted to 27°C in 12 h:12 h light:dark conditions for seven days before being assayed. Fly strains used in this study included: iso31, ClkJrk [17], timb [18], perb [19], Pdf-Gal4 [20], UAS-Clock [14], UAS-ClockRNAi (VDRC #42834), UAS-Aβ42ArcM [21], UAS-tim [22], UAS-dTRPA1 [23] andPdf [20].

**Triglyceride and protein measurements**

Fat bodies were dissected from abdomens of 4–7 d old mated females as described previously [24]. Fat bodies were homogenized in lysis buffer containing 140 mM NaCl, 50 mM Tris-HCl, pH 7.4, 0.1% Triton X and 1X protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany) and triglyceride and protein measurements were made using the triglyceride LiquiColor kit (Stanbio Laboratory, Boerne, TX) and bicinchoninic acid protein assay kit (Thermo Scientific, Waltham, MA), respectively, according to manufacturer’s instructions. Single balancer chromosomes were used to identify Gal4 and transgene only control flies for these experiments; since the single balancer chromosomes had no difference in triglycerides compared to wildtype chromosomes (data not shown), both wildtype and balancer chromosomes were denoted as “+” for clarity. Samples were collected at Zeitgeber time (ZT) 0, 4, 8, 12, 16, and 20 and averaged across all time points unless otherwise noted.

**Behavior measurements**

Flies were entrained in 12 h:12 h light:dark conditions for at least two days before assessing circadian behavior using the previously described DAMS system (Trikinetics, Waltham MA). The flies were maintained on 5% sucrose and 2% agar throughout the recording. Activity was collected in five-minute bins for a minimum of five days in constant darkness and analyzed using Clocklab software (Actimetrics) as described previously [22].

**Feeding measurements**

Food consumption was measured over a 24-hour period using a modified version of the CAFE assay as described [15]. Briefly, flies were entrained in 12 h:12 h light:dark conditions for at least two days on regular food at 25°C. On the day of the assay, flies were flipped into vials with 1% agar as a water source and 5% sucrose in 5 μl calibrated glass micropipettes (VWR, West Chester, PA) as their only food source. After 24 hours, the amount of liquid food consumed was measured and was adjusted to take into account evaporation, which was measured using a sucrose-filled micropipette in a 1% agar vial without flies.

**Results**

Fat body triglycerides are unchanged across the circadian day and in clock mutants

To investigate the role of the circadian system in regulating lipid accumulation in *Drosophila*, we started by measuring triglycerides at different times of day as many circadian-regulated processes proceed in a cyclic manner. In *Drosophila*, the majority of stored triglycerides are found in the fat body, an organ analogous to the mammalian liver and adipose tissue [25]. Thus, we measured triglyceride levels in abdominal fat bodies dissected from wildtype animals entrained to a 12 h:12 h light:dark cycle. In contrast to feeding that occurs rhythmically over the course of the day [15], fat body triglycerides remained constant at all time points tested (Fig. 1A).

Although fat body triglycerides fail to cycle, it is possible that the circadian clock controls overall lipid levels. To address this question, we measured triglycerides in fat bodies of clock mutant animals, ClkJrk, cyp10, tim10, and perb, and compared these to an isogenized w1118 wildtype control strain (iso31), a background into which all of the clock mutant strains were outcrossed at least 5 times. Triglycerides were similar in all of these animals suggesting that an absence of circadian clocks in all cells does not perturb fat body lipid levels (Fig. 1B).

Inhibiting the activity of the Clk transcription factor in PDF neurons increases triglycerides

We recently demonstrated that a peripheral clock in metabolic tissues promotes glycogen accumulation, while neuronal clocks oppose this action. Because of these opposing effects, circadian mutants that lack clocks in both metabolic and neuronal tissues have normal glycogen levels [15]. A similar regulation of lipid levels is suggested by the finding that disruption of the clock in metabolic tissues decreases triglycerides [15] while clock mutants have normal levels (Fig. 1B). To determine if the central clock in

Figure 1. Fat body triglycerides fail to cycle and are unaffected in circadian clock mutants. (A) Triglyceride/protein ratios of fat bodies dissected from 4–7 day old female iso31 (wildtype) flies at Zeitgeber time (ZT) 0, 4, 8, 12, 16, and 20. (B) Triglyceride/protein ratios of fat bodies dissected from 4–7 day old ClkJrk, cyp10, tim10, and perb females and compared to iso31 controls. Each experiment was performed at least three times with greater than 65 animals assayed in total, and values represent mean ± SEM. doi:10.1371/journal.pone.0019921.g001
particular affects fat body triglycerides, we used the Gal4/UAS system to specifically inhibit this clock. The central clock in flies is located in the ventral lateral neurons (LNvs), which specifically express the neuropeptide, pigment dispersing factor (PDF). As previously reported, expression of a dominant-negative form of the circadian transcription factor Clock (CLKΔ) in these neurons using Pdf-Gal4 inhibits the central clock [14]. Interestingly, ectopic expression of CLKΔ in LNvs led to an increase in fat body triglycerides, in particular when two copies of Pdf-Gal4 were used to express CLKΔ (Fig. 2A, 2B). A similar phenotype was observed when a ClkRNAi construct was expressed in these neurons using two copies of Pdf-Gal4 (Fig. 2C, 2D). While the expression of CLKΔ eliminates overt rhythms of rest:activity, the RNAi construct resulted only in a lengthening of circadian period, indicating that it preserves general clock function (Table 1, [14]). Nevertheless, there was a significant effect on lipid levels.

Figure 2. Inhibiting Clk gene function in the PDF neurons results in increased fat body triglycerides. (A) Triglyceride/protein ratios of fat bodies dissected from 4–7 day old Pdf-Gal4/UAS-ClkΔ and Pdf-Gal4/UAS-ClkΔ; Pdf-Gal4/+ female flies and compared to Pdf-Gal4/+; Pdf-Gal4/+ and UAS-ClkΔ/+ controls. (B) Triglyceride/protein ratios of fat bodies dissected from 4–7 day old Pdf-Gal4/UAS-ClkΔ; Pdf-Gal4/+ male flies and compared to Pdf-Gal4/+; Pdf-Gal4/+ controls. (C) Triglyceride/protein ratios of fat bodies dissected from 4–7 day old Pdf-Gal4/UAS-ClkΔ; Pdf-Gal4/+; UAS-ClkRNAi/+ and Pdf-Gal4/+; UAS-ClkRNAi/+; Pdf-Gal4/+; Pdf-Gal4/+; UAS-ClkRNAi/+ controls. (D) Triglyceride/protein ratios of fat bodies dissected from 4–7 day old Pdf-Gal4/+; Pdf-Gal4/+; UAS-ClkRNAi/+; Pdf-Gal4/+; UAS-ClkRNAi/+ controls. (E) Triglyceride/protein ratios of fat bodies dissected from 4–7 day old yw; Pdf-Gal4/+ female flies and compared to yw controls. (A,C,E) Each experiment was performed at least three times with greater than 100 animals in total and values represent mean ± SEM. *, # p<0.05 assessed by one-way ANOVA followed by post-hoc Tukey test compared to Pdf-Gal4 only and transgene only genotypes, respectively. (B,D) Each experiment was performed three times with greater than 40 animals in total, and the animals were dissected between ZT6-9. A representative experiment is shown and values represent mean ± SD. * p<0.05 assessed by Student’s t-test. doi:10.1371/journal.pone.0019921.g002
The neuropeptide PDF is not only a marker for the LNvs, it is also an important output of these neurons required for the maintenance of rest-activity rhythms [20]. PDF is positively regulated by the circadian transcription factors Clk and cry [26]; therefore, it is a good candidate for mediating the effects of CLK on triglycerides. However, triglyceride levels were normal in Pdfiso mutants (Fig. 2E), suggesting that other outputs are responsible for this effect. Together these data indicate that the central clock neurons affect lipid accumulation in the fat body, but do not do so through PDF.

Expression of a neurodegeneration-inducing protein in clock neurons results in elevated triglycerides

A physiological context during which normal circadian rhythms begin to break down is during aging. Specifically in Drosophila, the very robust locomotor activity rhythms decline as flies age [27]. Given that metabolic changes also occur with age, it is possible that triglyceride levels are affected by the aging process. To test this hypothesis, we aged wildtype iso flies 50–55 days and compared their fat body triglycerides to those of young one-week-old flies. The triglyceride levels of the aged flies trended to be higher than the young ones, although the difference did not reach statistical significance (Fig. 3A).

Our data indicate that normal aging does not have a profound effect on lipid accumulation. However, a number of age-related syndromes, such as neurodegenerative disorders, are associated with metabolic defects [28,29]. Drosophila has been used successfully to model neurodegenerative disorders by expressing pathogenic forms of disease-causing genes in the fly's nervous system [30]. Interestingly, when we expressed Aβ42ArcM, a pathogenic form of the amyloid protein shown to induce neurodegeneration [31], in the central clock neurons using Pdf-Gal4, fat body triglycerides were elevated similarly to when CLK and CIRNAi were expressed in the same neurons (Fig. 3B). No circadian behavioral phenotype was observed in the Aβ42ArcM-expressing animals, suggesting that some function of the ventral lateral neurons, but not necessarily the clock in them, is important for regulating fat body triglycerides (Table 1).

To further address the role of the clock in PDF neurons in the control of fat body lipid storage, we sought to measure triglycerides in response to other genetic manipulations that alter activity rhythms. Our lab showed previously that preventing tim cycling by over-expressing it in all clock neurons abolishes behavioral rhythms [22]. Over-expression of tim in PDF neurons alone does not eliminate rhythms altogether, but leads to an increased incidence of arrhythmia (Table 1); however, no change in fat body triglycerides was observed (Fig. 4A). Additionally, no lipid phenotype was observed when the heat-sensitive Na+ channel, dTRPA1, was used to increase excitability of the PDF neurons (Fig. 4B), even though this manipulation resulted in an arrhythmic behavioral rhythm phenotype, similar to that seen when the bacterial Na+ channel, NaChBac was expressed in the same neurons (Table 1, [31]). Together these data suggest that altering the function of the PDF neurons leads to increased fat body triglycerides, but the circadian function of these neurons may not be important for this purpose.

Increased feeding does not account for the increased lipid phenotype observed when PDF neuron function is altered

In addition to the storage of glycogen and lipids, feeding behavior is controlled by the circadian system [7,15]. While recent evidence points to the fat body clock as a major circadian regulator of feeding in Drosophila, the contribution of the central clock to controlling feeding has not been addressed. Therefore, it is possible that the increased fat phenotype observed above is a result of increased feeding. To test this hypothesis, we measured food consumption over a 24-hr period in animals expressing CLKΔ, CIRNAi, and Aβ42ArcM in the PDF neurons. The total food intake of these animals was not higher than that of controls; in fact, the CIRNAi flies had less food consumption than controls (Fig. 5), indicating that increased feeding does not account for the high lipids observed in animals with altered clock neurons.

**Discussion**

In this study, we established a role for the PDF circadian neurons in controlling lipid storage non-cell-autonomously in the fat body. The effects on lipid levels reported here are consistent with our previously published data on the control of glycogen levels by neuronal clocks [15]. In addition, we have identified specific neurons – those that house the central clock – that account for at least some of the neuronal effects on triglycerides. Given this apparent control of lipid metabolism by the circadian neurons, one might expect that fat body triglycerides would cycle throughout the PDF expressing animals.

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**Table 1. Activity rhythms in flies expressing ClkRNAi, Aβ42ArcM, tim, and dTRPA1 in PDF neurons.**

| Genotype | Number tested (% arrhythmic) | Period Length (hrs ± SD) | FFT (± SD) |
|----------|-----------------------------|--------------------------|------------|
| Pdf-Gal4/+; Pdf-Gal4/+ | 130 (18.5) | 23.8±0.4 | 0.093±0.1 |
| UAS-CIRNAi/+ | 45 (15.6) | 24.0±0.6 | 0.069±0.04 |
| UAS-Aβ42ArcM/+ | 48 (8.3) | 24.2±0.4 | 0.065±0.03 |
| Pdf-Gal4/+; Pdf-Gal4->ClkRNAi | 44 (4.5) | 24.9±0.4* | 0.081±0.04 |
| Pdf-Gal4/+; Pdf-Gal4->Aβ42ArcM | 44 (18) | 24.2±0.5 | 0.058±0.03 |
| Pdf-Gal4/+; tim; Pdf-Gal4/+ | 47 (36.2) | 24.3±0.6 | 0.055±0.04 |
| UAS-dTRPA1/+ | 48 (27.1) | 24.0±0.3 | 0.102±0.04 |
| UAS-dTRPA1/+; Pdf-Gal4/+ | 47 (12.8) | 23.6±0.3 | 0.126±0.06 |
| UAS-dTRPA1/+; Pdf-Gal4/+ | 43 (95.3) | 24.7±1.8 | 0.054±0.02 |

Activity rhythm data of 1–2 week old female flies.

*Data were collected from flies that were housed at 27°C throughout the entire experiment.

*p<0.05 assessed by one-way ANOVA followed by post-hoc Tukey test compared to Pdf-Gal4 only and UAS-CIRNAi genotypes.

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the circadian day, but data presented here show this is not the case. One possibility is that triglyceride cycling occurs, but cannot be detected with current methodology. Another possibility is that regulation of fat body triglycerides by multiple clocks prevents cyclic control that may be exerted by one clock alone. This would allow for maintenance of constant levels, which may be more beneficial for the organism. Finally, as noted below, the current study favors a non-circadian effect of the LNvs on fat body triglycerides, which would also explain the lack of cycling.

The dominant-negative CLK protein data, and even the RNAi knockdown data, implicate the circadian clock in controlling lipid storage. This would be consistent with previous reports on the control of lipid metabolism by the circadian clock in mammals [6,7]. However, we elicit a similar triglyceride increase when the Alzheimer’s Disease-causing protein Aβ42ArcM is expressed in the PDF neurons, and this manipulation has no effect on the central clock as evidenced by normal activity behavior (Table 1). Conversely, expression of tim and dTRPA1 in the clock neurons affects circadian function by altering activity rhythms (Table 1), but has no effect on fat body triglycerides (Fig. 4). While it is possible that tim over-expression in PDF neurons alone is not strong enough to affect triglycerides (the effect on behavioral rhythms in this case is weaker than that of tim over-expression by the tim-Gal4 driver [22]), and that dTRPA1 affects specific outputs from the neurons rather than the clock itself, a more likely scenario is that the circadian clock is not involved in eliciting the triglyceride phenotype described in this study. The effect of Clk shown here would then reflect a non-circadian function of this

Figure 3. The effects of age and neurodegeneration on fat body triglycerides. (A) Triglyceride/protein ratios of fat bodies dissected from 50–55 day old wild type females (old iso31) at ZT8 and compared to 4–7 day old wild type females (young iso31) at ZT8. (B) Triglyceride/protein ratios of fat bodies dissected from 4–7 day old Pdf-Gal4/+; UAS-Aβ42ArcM/+ and Pdf-Gal4/+; UAS-Aβ42ArcM/+ animals and compared to Pdf-Gal4/+; UAS-Aβ42ArcM/+ and UAS-Aβ42ArcM/+ controls. Each experiment was performed at least three times and values represent mean ± SEM. The data in (A) were obtained from 36 animals and the data in (B) from greater than 100 animals.

*, # p<0.05 assessed by one-way ANOVA followed by post-hoc Tukey test compared to Pdf-Gal4 only and transgene only genotypes, respectively.

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Figure 4. Expression of tim and dTRPA1 in the PDF neurons has no effect on fat body triglycerides. (A) Triglyceride/protein ratios of fat bodies dissected from 4–7 day old Pdf-Gal4/UAS-tim and Pdf-Gal4/UAS-tim/+ female flies and compared to Pdf-Gal4/+; UAS-Aβ42ArcM/+ controls. Animals from each genotype were dissected at ZT8 and values in the two time points were averaged. (B) Triglyceride/protein ratios of fat bodies dissected from 7–10 day old Pdf-Gal4/UAS-dTRPA1; Pdf-Gal4/+ female flies at ZT 0–2 and compared to Pdf-Gal4/+; Pdf-Gal4/+ controls at ZT 0–2. Each experiment was performed at least three times and values represent mean ± SEM. The data in (A) were obtained from 120 animals and the data in (B) from 45 animals for each genotype.

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gene. Additionally, the major circadian output signal of LNvs, PDF, does not appear to be involved in the control of fat body lipids either as Pdf01 mutants have normal triglycerides (Fig. 2E). This is consistent with a recent study demonstrating that a PDF-independent factor mediates part of the complex rhythm observed when the electrical activity of the LNvs is altered [32].

One outstanding question raised by this study is how the central clock neurons communicate with the fat body to regulate lipid metabolism. One possibility is that the circadian neurons regulate overall feeding behavior, which would then secondarily affect lipid storage. However, this mechanism seems unlikely, as expressing CLKA, GßRNAi or AßH2ArcM in the PDF neurons increases fat body triglycerides but doesn’t increase the amount of food consumed over a 24-hr period (Figs. 2, 3, 5). Another potential mechanism is that the fat body receives signals from the brain to control lipid metabolism through direct neuronal connections. In mammals, the sympathetic nervous system innervates white adipose tissue and is thought to be a major initiating stimulus for lipid mobilization [33,34]. A similar circuit might be present in Drosophila, although whether the fat body is innervated by the fly’s nervous system is still unknown. A third possibility is that the PDF neurons or another neuronal population that receives signals from the PDF neurons produces a secreted factor that travels through the hemolymph and acts on the fat body to regulate lipid levels. While it seems that PDF is dispensable for controlling lipid levels since Pdf01 mutants have normal triglycerides (Fig. 2E), other peptides are expressed in the fly brain such as the insulin-like peptides (DILPs), the feeding peptide neuropeptide F (NPF), and a number of novel peptides whose functions are still unclear [35,36,37]. Recent studies showed that the PDF neurons express NPF, which based upon the role of NPF in regulating feeding and metabolism, could be a potential mediator of the lipid storage phenotype described here [36,39,39]. In any case, this study implicates the central clock neurons in controlling fat body triglycerides and demonstrates the utility of the Drosophila system to increase our understanding of the mechanisms whereby specific populations of neurons regulate lipid metabolism.

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Author Contributions
Conceived and designed the experiments: JRD AS. Performed the experiments: JRD RE AC. Analyzed the data: JRD RE AC AS. Contributed reagents/materials/analysis tools: JRD RE AC AS. Wrote the paper: JRD AS.

References

1. Stein CJ, Colditz GA (2004) The epidemic of obesity. J Clin Endocrinol Metab 89: 2522–2525.
2. Kohlwein SD (2010) Triacylglycerol homeostasis: insights from yeast. J Biol Chem 285: 15663–15667.
3. Benarroch EE (2010) Neural control of feeding behavior: Overview and clinical correlations. Neurology 74: 1643–1650.
4. Nogueiras R, Lopez M, Dieguez C (2010) Regulation of lipid metabolism by energy availability: a role for the central nervous system. Obes Rev 11: 183–201.
5. Sach-Parys K, Lombardelli S, Khan EZ, McDowall K, An-Yong IT, et al. (2000) Neural connections of hypothalamic neuroendocrine nuclei in the rat. J Neuroendocrinol 12: 635–648.
6. Rodie RD, McNamara P, Curtis AM, Boston RC, Panda S, et al. (2004) BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. PLoS Biol 2: e377. doi:10.1371/journal.pbio.0020377.
7. Turek FW, Joshi C, Kolmack A, Lin E, Ivanova G, et al. (2005) Obesity and metabolic syndrome in circadian Clock mutant mice. Science 308: 1043–1045.
8. Damlova F, Le Minh N, Preti\ rror;ner N, Kornmann B, Fleury-Olela F, et al. (2000) Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. Genes Dev 14: 2950–2961.
9. Le Martelot G, Claudel T, Garfield D, Schaad O, Kornmann B, et al. (2009) REV-ERBa/Ralpha participates in circadian SREBP signaling and bile acid homeostasis. PLoS Biol 7: e1000181. doi:10.1371/journal.pbio.1000181.
10. Vollmers C, Gill S, DiTuccio L, Palvarthy SR, Le HD, et al. (2009) Time of feeding and the intrinsic circadian clock drive rhythms in hepatic gene expression. Proc Natl Acad Sci U S A 106: 21453–21458.
11. Baker KD, Thummel CS (2007) Diabetic larvae and obese flies-emerging studies of metabolism in Drosophila. Cell Metab 6: 257–266.
23. Hamada FN, Rosenzweig M, Kang K, Pulver SR, Ghezzi A, et al. (2008) An internal thermal sensor controlling temperature preference in Drosophila. Nature 454: 217–220.

24. DiAngelo JR, Birnbaum MJ (2009) Regulation of fat cell mass by insulin in Drosophila melanogaster. Mol Cell Biol 29: 6341–6352.

25. Hoshizaki DK (2005) Fat-Cell Development. In: Gilbert LI, Iatrou K, Gill SS, eds. Comprehensive Molecular Insect Science. edited by Amsterdam/New York: Elsevier. pp 315–345.

26. Park JH, Hoffrich-Forster C, Lee G, Liu L, Rosbash M, et al. (2000) Differential regulation of circadian pacemaker output by separate clock genes in Drosophila. Proc Natl Acad Sci U S A 97: 3608–3613.

27. Koh K, Evans JM, Hendrickx JC, Schgal A (2006) A Drosophila model for age-associated changes in sleepwake cycles. Proc Natl Acad Sci U S A 103: 13943–13947.

28. Bjorkhem I, Leoni V, Meaney S (2010) Genetic connections between neurological disorders and cholesterol metabolism. J Lipid Res 51: 2489–2503.

29. de la Monte SM, Longato L, Tong M, Wandji JR (2009) Insulin resistance and neurodegeneration: roles of obesity, type 2 diabetes mellitus and non-alcoholic steatohepatitis. Curr Opin Investig Drugs 10: 1049–1060.

30. Lessing D, Bonini NM (2009) Maintaining the brain: insight into human neurodegeneration from Drosophila melanogaster mutants. Nat Rev Genet 10: 359–370.

31. Nitabach MN, Wu Y, Sheeba V, Lemon WC, Strumbos J, et al. (2006) Electrical hyperexcitation of lateral ventral pacemaker neurons desynchronizes down-stream circadian oscillators in the fly circadian circuit and induces multiple behavioral periods. J Neurosci 26: 479–489.

32. Sheeba V, Sharma VK, Gu H, Chou YT, O'Dowd DK, et al. (2008) Pigment dispersing factor-dependent and -independent circadian locomotor behavioral rhythms. J Neurosci 28: 217–227.

33. Bartness TJ, Bamsah M (1998) Innervation of mammalian white adipose tissue: implications for the regulation of total body fat. Am J Physiol 275: R1399–1411.

34. Bartness TJ, Song CK (2007) Thematic review series: adipocyte biology. Sympathetic and sensory innervation of white adipose tissue. J Lipid Res 48: 1653–1672.

35. Rulifson EJ, Kim SK, Nuse R (2002) Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. Science 296: 1118–1120.

36. Wu Q, Wen T, Lee G, Park JH, Cai HN, et al. (2003) Developmental control of foraging and social behavior by the Drosophila neuropeptide Y-like system. Neuron 39: 147–161.

37. Verleyen P, Baggerman G, Wiehart U, Schoeters E, Van Lommel A, et al. (2004) Expression of a novel neuropeptide, NVGTLARDFQFLPPNamide, in the larval and adult brain of Drosophila melanogaster. J Neurochem 88: 311–319.

38. Kula-Eversole E, Nagoshi E, Shang Y, Rodriguez J, Alaba R, et al. Surprising gene expression patterns within and between PDF-containing circadian neurons in Drosophila. Proc Natl Acad Sci U S A 107: 13497–13502.

39. Lee G, Bahn JH, Park JH (2006) Sex- and clock-controlled expression of the neuropeptide F gene in Drosophila. Proc Natl Acad Sci U S A 103: 12580–12585.