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The splicing factor transformer2 (tra2) functions in the *Drosophila* fat body to regulate lipid storage

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Abstract

Excess nutrients are stored as triglycerides mainly in the adipose tissue of an animal and these triglycerides are located in structures called lipid droplets. Previous genome-wide RNAi screens in *Drosophila* cells identified splicing factors as playing a role in lipid droplet formation. Our lab has recently identified the SR protein, 9G8, as an important factor in fat storage as decreasing its levels results in augmented triglyceride storage in the fat body. Previous *in vitro* studies have implicated 9G8 in the regulation of splicing of the sex determination gene *doublesex* (*dsx*) by binding to transformer (*tra*) and transformer2 (*tra2*); however, any function of these sex determination proteins in regulating metabolism is unknown. In this study, we have uncovered a role of *tra2* to regulate fat storage *in vivo*. Inducing *tra2*^{dsRNA} in the adult fat body resulted in an increase in triglyceride levels but had no effect on glycogen storage. Consistent with the triglyceride phenotype, *tra2* knockdown flies lived longer under starvation conditions. In addition, this increase in triglycerides is due to more fat storage per cell and not an increase in the number of fat cells. Interestingly, the splicing of *CPT1*, an enzyme involved in the breakdown of lipids, was altered in flies with decreased *tra2*. The less-catalytically active isoform of CPT1 accumulated in *tra2*^{dsRNA} flies suggesting a decrease in lipid breakdown, which is consistent with the increased triglyceride levels observed in these flies. Together, these results suggest a link between mRNA splicing, sex determination and lipid metabolism and may provide insight into the mechanisms underlying tissue-specific splicing and nutrient storage.

Keywords

Drosophila; lipids; fat body; CPT1; *tra2*

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1. Introduction

As animals consume food, excess nutrients are stored as triglycerides mainly within adipose tissue in structures known as lipid droplets. Such storage is the result of an evolutionary adaptation that allowed species to endure famines and plagues. As food becomes easily accessible in today's society and people lead sedentary lifestyles, negative consequences of overconsumption and excess nutrient storage are becoming increasingly apparent. In the United States, excessive fat storage has resulted in complications that account for some of the leading causes of death such as heart disease and stroke [1], with rates of childhood and adult obesity on the rise [2]. Understanding the biology that controls how our bodies metabolize and store excess nutrients can help both combat the increasing rates of obesity and prevent obesity-related diseases such as diabetes and cardiovascular disease.

Drosophila are organisms with modest dietary and spatial needs, they have a short generation time, and can be propagated at a fairly low cost [3]. *Drosophila* have a simple nervous system, and store triglycerides and glycogen in a liver and adipose-like organ (called the fat body), both of which share functional similarities to those of humans. The genes regulating glycogen and lipid storage in these organs within *Drosophila* are highly analogous to those of humans, coding for proteins such as insulin, glucagon, and lipases [4]. Due to all of these similarities, *Drosophila* provide an ideal system to study the molecular control of energy metabolism, more specifically how fat storage is regulated within their fat body.

In order to better understand genes important for triglyceride storage in *Drosophila*, genome-wide RNA interference (RNAi) screens have been performed and identified genes which, when disrupted, lead to alterations in lipid droplet size and/or number [5, 6]. One interesting family of genes, which resulted in smaller, more dispersed lipid droplets when their expression is decreased, included genes involved in RNA processing. However, whether these RNA processing genes regulated lipid storage *in vivo* was not known. To further understand the function of these genes in regulating lipid storage *in vivo*, we have decreased the expression of a number of splicing proteins specifically in the fly fat body and measured triglycerides. In addition to identifying members of the U1 and U2 snRNP as being important for lipid storage, we also identified an SR protein called 9G8/SFRS7 that is important for triglyceride storage in the *Drosophila* fat body [7]. This phenotype can be explained by altered splicing of a lipid metabolic gene important for β -oxidation of fatty acids known as *CPT1*. In *Drosophila*, the *CPT1* gene has two isoforms resulting from a mutually exclusive alternate sixth exon (exon 6A or 6B). The product of isoforms containing exon 6A exhibits higher enzyme activity than those containing exon 6B, leading to increased lipid breakdown [8]. Interestingly, while wildtype flies had more *CPT1* that included exon 6A, flies with decreased fat body 9G8 expressed higher levels of the *CPT1* isoform containing exon 6B [7]. The presence of more exon 6B-containing *CPT1* isoforms results in less *CPT1* enzyme activity, which is consistent with the augmented triglyceride stores observed in these flies. Identifying the metabolic functions of 9G8 raised the question as to whether other RNA splicing proteins were involved in the regulation of fat metabolism. Previous studies have shown that 9G8 binds to two RNA-binding proteins called transformer (tra) and transformer2 (tra2) to correctly process the *doublesex* (*dsx*) gene, which is

important for controlling sex determination [9]. While *tra* and *tra2* have been well characterized in the regulation of sex determination, any function of these proteins in controlling metabolism is unknown.

In this study, we characterized the metabolic function of *tra2* in the *Drosophila* fat body. Similar to the 9G8 phenotype, decreasing *tra2* levels in the *Drosophila* fat body using RNAi resulted in increased starvation resistance and a large increase in triglycerides. This increased storage of triglycerides seemed to be due to an increase in the amount of fat stored per cell and not due to increased food consumption. In addition, the splicing of *CPT1* was altered in flies with decreased *tra2*, resulting in a higher amount of *CPT1* isoforms containing exon 6B, which are less catalytically active, potentially explaining the increased triglyceride levels. Together, the results of this study provide *in vivo* evidence for a connection between *tra2*-regulated splicing and lipid metabolism.

2. Materials and Methods

2.1 Fly Genetics

The following fly lines were used in this study: *yolk-Gal4* [10], *y[1] v[1]*; *P{y[+t7.7]=CaryP}attP2* (referred to as *atp2*; BL#36303), and *y[1] v[1]*; *P{y[+t7.7] v[+t1.8]=TRiP.JF02852}attP2* (referred to as *tra2^{dsRNA}*; BL#28018). Flies were grown at 25°C on a 12 hour:12 hour light:dark cycle on a standard cornmeal-sugar yeast medium (9 g *Drosophila* agar (Genesee Scientific), 100 mL Karo Lite Corn Syrup, 65 g cornmeal, 40 g sucrose, and 25 g whole yeast in 1.25 L water).

2.2 Macromolecule Assays

Two approximately 1-week-old females or three fat bodies dissected from these animals were homogenized in lysis buffer containing 140 mM NaCl, 50 mM Tris-HCl, pH 7.4, 0.1% Triton X, and 1× protease inhibitor cocktail (Roche). Triglycerides were measured using the Infinity Triglyceride Reagent (ThermoFisher Scientific), proteins were measured using the Pierce BCA Assay kit (ThermoFisher Scientific), and DNA content was measured using the Quant-iT DNA Assay kit (Invitrogen) according to manufacturer's instructions. To measure glycogen levels, homogenized samples were treated with 8 mg/mL amyloglucosidase (Sigma-Aldrich) in 0.2M citrate buffer, pH 5.0 for 2 hours at 37°C. Total glucose was then measured using the Pointe Scientific Glucose Oxidase Kit (ThermoFisher Scientific). A similar procedure was performed for samples that were not treated with amyloglucosidase in order to measure free glucose. Final glycogen levels were determined by subtracting free glucose from total glucose.

2.3 Feeding Assay

Food consumption was measured over a 24-hour period by using a modified version of the Capillary Feeder (CAFÉ) Assay as described previously [11]. Three 1-week old adult female flies were placed in each vial containing 1% agar. A 5% sucrose solution was provided via a glass capillary tube as the sole food source. The amount of sucrose left was measured after 24 hours elapsed. Identical vials, with no flies in them, were used in order to correct for any evaporation that may have occurred during the experiment.

2.4 Starvation Resistance

Approximately 1-week-old female flies were placed in vials containing only a water source in the form of 1% agar. The number of surviving flies was measured every 6 hours until all flies were dead. The collected data was analyzed via Online Application for Survival Analysis (OASIS (<https://sbi.postech.ac.kr/oasis2/>)).

2.5 RNA Isolation

Fat bodies dissected from approximately fifteen 1-week old adult females were homogenized in 1 ml Ribozol (Amresco). The sample was incubated at room temperature for 5 minutes (min) and was then centrifuged at 12,000 rpm for 15 min at 4°C. The sample was chloroform extracted, incubated at room temperature again for 5 min, and then centrifuged at 12,000 rpm for 15 min at 4°C. RNA was then precipitated by adding an equal volume of isopropanol to the sample and centrifuging at 12,000 rpm for 15 min at 4°C. The pellet was then washed with 70% ethanol. Ethanol was removed from the sample, which then was left to air dry for 5 min. The pellet was re-suspended in RNase free water.

2.6 DNase Treatment, cDNA Synthesis and qPCR

5 µg of total RNA was treated with DNase using the DNA-Free turbo kit according to manufacturer's protocol (Life Technologies). 0.25 µg of DNase-treated RNA was reverse transcribed using qScript XLT cDNA SuperMix (Quanta Biosciences) according to manufacturer's protocol. cDNA product was amplified via qPCR using 1 µL cDNA, 1X PowerUp SYBR Green Master Mix (Applied Biosystems), and 0.2 µM primers in a 25 µL reaction. Samples were placed in a Step-One Plus qPCR machine starting with an incubation for 2 min at 50°C and 2 min at 95°C followed by 40 cycles of 30 sec at 95°C, 60 sec at 60°C, and 30 sec at 72°C. After the cycling conditions, a melt curve was performed. The primers used for exon 6A-containing CPT1 were: (Forward: 5'CCGCTGGTTTGACAAGTG3', Reverse: 5'TCATCGACGATCAGGTTCTC3') [8]. Primers used for exon 6B-containing CPT1 were: (Forward: 5'AATGGTCGCGTTGGCTTC3', Reverse: 5'TCCCAAACCGGTGCATC3') (Price et al., 2010). Primers used for rp49 were: (Forward: 5'GACGCTTCAAGGGACAGTATCTG3', Reverse: 5'AAACGCGGTTCTGCATGAG3'). Primers used for tra2 were: (Forward: 5'GAACATCCACAAGCAAGCCG3', Reverse: 5'ATACGGCGACCATCCACTTC3'). The relative expression of *CPT1* and *tra2* were normalized by dividing by *rp49* expression levels.

2.7 Statistics

A Student's t-test was performed to compare the *yolk-Gal4>tra2^{dsRNA}* genotype to the *yolk-Gal4>atp2* controls for all assays performed in this study except starvation resistance. The Kaplan-Meier Estimator was used in OASIS to analyze survival data during starvation. The Log Rank test was used to determine differences in mean lifespan while on starvation medium and the Fisher's Exact test was used to determine differences in 50% survival. P-values <0.05 were considered significant for all statistical analyses.

3. Results

3.1 Tra2 regulates triglyceride storage in the *Drosophila* fat body

To examine the potential metabolic functions of the splicing factor *tra2*, we decreased *tra2* levels specifically in the female fat body by using the Gal4-UAS system to induce RNAi knockdown of *tra2*. This approach was effective because relative *tra2* mRNA levels in *yolk-Gal4>tra2^{dsRNA}* flies were lower than that of *yolk-Gal4>atp2* control flies (Fig. 1A). When working with flies with decreased *tra2*, we observed that they had an enlarged abdomen (Fig. 1B), suggesting some metabolic or nutrient storage function of *tra2*. To begin addressing whether the *yolk-Gal4>tra2^{dsRNA}* had any metabolic abnormalities, we wanted to test whether these flies could respond normally to starvation, a nutrient stress. Interestingly *yolk-Gal4>tra2^{dsRNA}* flies exhibited significantly longer survival rates on starvation media when compared to the control flies (Fig. 1C), suggesting a metabolic function of *tra2* in the fly fat body.

Consistent with enhanced starvation resistance, decreasing the expression of *tra2* in the fat body also results in increased triglyceride storage in female flies compared to the *atp2* controls (Fig. 2A). However, glycogen storage was not altered in *tra2*-RNAi flies (Fig. 2B). Together, these findings indicate that the splicing protein *tra2* functions in the regulation of lipid homeostasis in the *Drosophila* fat body resulting in an enlarged abdomen, increased triglyceride levels and enhanced resistance to starvation.

3.2 Tra2 regulates the amount of lipid stored in each fat cell

The increased triglyceride storage phenotype observed in flies with decreased *tra2* levels may result from an increased number of fat cells within the fat body, an increased amount of fat stored per cell, or both. As the number of cells in an organ increases, the total DNA content of that organ also increases, allowing DNA content to be used as a surrogate measurement for cell number [12]. We hypothesize that the number of fat cells present in *tra2*-RNAi flies would be increased accounting for the augmented triglyceride storage phenotype. Surprisingly, we observed that the amount of DNA is actually decreased in *tra2*-RNAi flies (Fig. 3A) suggesting that the increased triglyceride phenotype in flies with decreased *tra2* expression is not due to an increase in fat cell number. To test whether the amount of fat stored per cell was affected by decreasing *tra2*, triglyceride/DNA ratios were also measured (Fig. 3B). An increase in the triglyceride/DNA ratio was observed in *tra2*-RNAi flies, indicating that *tra2* regulates lipid homeostasis by controlling the amount of fat stored per fat body cell.

3.3 Tra2 controls the splicing of CPT1

One way in which triglyceride storage could be augmented in the *tra2*-RNAi flies is by increasing food intake. In order to test this hypothesis, food consumption was measured over a 24-hour period using capillary feeding (CAFÉ) assays [11]. Monitoring food intake showed that *yolk-Gal4>tra2^{dsRNA}* adult female flies were not consuming more food than the *yolk-Gal4>atp2* control flies (Fig. 4A). These results indicate that the augmented fat storage in *tra2*-RNAi flies was not caused by increasing feeding.

Since *tra2*-RNAi flies have more triglycerides stored in each cell compared to the control animals, we hypothesized that *tra2* may control the processing of genes important for lipid synthesis or breakdown. We decided to investigate the processing of *CPT1*, the rate-limiting step of β -oxidation. *Drosophila* contain two forms of *CPT1* resulting from the inclusion of an alternate sixth exon (6A or 6B) (Fig. 4B). *CPT1* enzymes containing exon 6A have higher activity than those including 6B [8]. Therefore, it is possible that flies with decreased *tra2* may have altered *CPT1* splicing causing triglycerides to accumulate. In order to investigate whether increased triglyceride levels in *tra2*-RNAi flies are caused by alternative splicing of *CPT1* resulting in accumulation of the less active form of the enzyme, we performed qPCR for the presence of these two alternative sixth exons of *CPT1* in flies with decreased *tra2*. Control flies produced more *CPT1* transcripts including exon 6A than 6B, while flies with less *tra2* in their fat bodies produced more *CPT1* transcripts including exon 6B than 6A exon (Fig. 4C), suggesting that *tra2*-RNAi flies may be undergoing less β -oxidation, thus contributing to the triglyceride storage phenotype. Together, these data suggest that *tra2* functions to control the splicing of *CPT1*, regulating the overall storage of triglycerides in the fly fat body.

4. Discussion

In this study, we set out to determine whether the splicing factor *tra2* functions in the *Drosophila* fat body to regulate lipid metabolism. We have shown that fat body-specific knockdown of *tra2* resulted in increased triglyceride storage and starvation resistance. The augmented storage of triglycerides seemed to be due to an increase in the amount of fat stored per cell and not to increased food consumption. Interestingly, we found that the splicing of *CPT1* was altered in flies with decreased *tra2*, resulting in a higher amount of the less active enzyme, potentially explaining the lipid storage phenotype.

Previous genome-wide RNAi screens identified a group of genes whose knockdown resulted in smaller, more dispersed droplets [5, 6]. This group included genes such as *SmB*, *SmD* and *prp8*, which are components of various snRNPs in the spliceosome, used for the removal of introns [13]. The effects of altered expression of snRNP proteins on lipid storage in cultured cells is consistent with previous studies performed by our lab showing that fat body knockdown of components of the U1 and U2 snRNPs also results in decreased triglyceride levels [5–7]. Interestingly, altering the expression of SR proteins has different metabolic effects than snRNP gene knockdown as knocking down the SR protein 9G8 in the fat body results in an increase in triglyceride storage [7]. It has also previously been shown that 9G8 binds to the splicing factor *tra2* and another RNA-binding protein called transformer (*tra*) to correctly process the *doublesex* (*dsx*) gene, which is important for controlling whether a fly develops into a male or female [9, 14]. Since 9G8 and *tra2* act together to regulate sex determination, it is possible that they play similar roles in controlling other processes such as lipid storage and the results described here support that hypothesis.

Beyond its function in the splicing of *dsx* to control sex determination, we have uncovered a new role for *tra2* in the splicing of the gene coding for the metabolic enzyme *CPT1*. We show here that decreasing *tra2* results in the alternative splicing of the gene for *CPT1*, an enzyme important for lipid breakdown. In *tra2* knockdown flies, the inclusion of the

alternate sixth exon is altered, resulting in more transcripts that include exon 6B causing the less active *CPT1* enzyme to accumulate [8]. This suggests that *tra2* may be binding directly to the *CPT1* pre-mRNA to regulate the alternative splicing of exons 6A and 6B in the mature *CPT1* transcript. This *CPT1* splicing phenotype is very similar to the *CPT1* splicing defect observed in flies with 9G8 decreased in the fly fat body [7]. Therefore, it is also possible that instead of binding directly to the *CPT1* transcript and regulating its alternative splicing, *tra2* could form a heterodimer with 9G8 and the entire complex binds to the *CPT1* transcript to control its processing. Identifying 9G8 and *tra2* binding sequences in the *CPT1* pre-mRNA will help clarify these possibilities.

In addition to regulating the splicing of *CPT1*, it is possible that *tra2* may regulate the splicing of additional lipid metabolic genes such as fatty acid synthase (*dFAS*) and acetyl-CoA carboxylase (*dACC*) which promote the synthesis of fatty acids or *brummer* (*bmm*), the fly homolog of adipose triglyceride lipase that is involved in lipolysis [4]. Each of these genes has multiple isoforms, but how the expression of these variants is regulated and whether these different isoforms have different enzyme activities is not known. Experiments designed to measure the splicing of these genes coding for enzymes important for lipid metabolism in *tra2*-RNAi flies will help provide insight into additional metabolic functions of *tra2*.

In summary, these findings show that the knockdown of *tra2* in the *Drosophila* fat body results in an increased triglyceride phenotype, which could be explained in part by altered splicing of the gene coding for the lipid breakdown enzyme, *CPT1*. These findings are consistent with previous studies analyzing the SR protein, 9G8, providing support of the hypothesis that these two RNA-binding proteins may be functioning together to control the splicing of lipid metabolic genes and therefore overall lipid homeostasis. The increased triglyceride storage seen with the knockdown of the human *tra2* homolog, *SFRS10* [15], has also been consistent with our results suggesting that *tra2* and other RNA binding proteins play a highly conserved role in regulating the expression and processing of important metabolic enzymes, many of which have yet to be identified. Together, these results suggest a link between mRNA splicing, sex determination and lipid metabolism and may provide insight into the mechanisms underlying tissue-specific splicing and nutrient storage and the development of obesity.

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References

1. Scully T. Diabetes in numbers. *Nature*. 2012; 485:S2–3. [PubMed: 22616094]
2. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011–2012. *JAMA*. 2014; 311:806–814. [PubMed: 24570244]
3. Stocker H, Gallant P. Getting started: an overview on raising and handling *Drosophila*. *Methods Mol Biol*. 2008; 420:27–44. [PubMed: 18641939]

4. Baker KD, Thummel CS. Diabetic larvae and obese flies-emerging studies of metabolism in *Drosophila*. *Cell Metab*. 2007; 6:257–266. [PubMed: 17908555]
5. Beller M, Sztalryd C, Southall N, Bell M, Jackle H, Auld DS, Oliver B. COPI complex is a regulator of lipid homeostasis. *PLoS Biol*. 2008; 6:e292. [PubMed: 19067489]
6. Guo Y, Walther TC, Rao M, Stuurman N, Goshima G, Terayama K, Wong JS, Vale RD, Walter P, Farese RV. Functional genomic screen reveals genes involved in lipid-droplet formation and utilization. *Nature*. 2008; 453:657–661. [PubMed: 18408709]
7. Gingras RM, Warren ME, Nagengast AA, Diangelo JR. The control of lipid metabolism by mRNA splicing in *Drosophila*. *Biochem Biophys Res Commun*. 2014; 443:672–676. [PubMed: 24333419]
8. Price NT, Jackson VN, Muller J, Moffat K, Matthews KL, Orton T, Zammit VA. Alternative exon usage in the single CPT1 gene of *Drosophila* generates functional diversity in the kinetic properties of the enzyme: differential expression of alternatively spliced variants in *Drosophila* tissues. *J Biol Chem*. 2010; 285:7857–7865. [PubMed: 20061394]
9. Sciabica KS, Hertel KJ. The splicing regulators Tra and Tra2 are unusually potent activators of pre-mRNA splicing. *Nucleic Acids Res*. 2006; 34:6612–6620. [PubMed: 17135210]
10. Georgel P, Naitza S, Kappler C, Ferrandon D, Zachary D, Swimmer C, Kopczynski C, Duyk G, Reichhart JM, Hoffmann JA. *Drosophila* immune deficiency (IMD) is a death domain protein that activates antibacterial defense and can promote apoptosis. *Dev Cell*. 2001; 1:503–514. [PubMed: 11703941]
11. Ja WW, Carvalho GB, Mak EM, de la Rosa NN, Fang AY, Liong JC, Brummel T, Benzer S. Prandiology of *Drosophila* and the CAFE assay. *Proc Natl Acad Sci U S A*. 2007; 104:8253–8256. [PubMed: 17494737]
12. DiAngelo JR, Birnbaum MJ. Regulation of fat cell mass by insulin in *Drosophila melanogaster*. *Mol Cell Biol*. 2009; 29:6341–6352. [PubMed: 19822665]
13. Matera AG, Wang Z. A day in the life of the spliceosome. *Nat Rev Mol Cell Biol*. 2014; 15:108–121. [PubMed: 24452469]
14. Lynch KW, Maniatis T. Assembly of specific SR protein complexes on distinct regulatory elements of the *Drosophila* doublesex splicing enhancer. *Genes Dev*. 1996; 10:2089–2101. [PubMed: 8769651]
15. Pihlajamäki J, Lerin C, Itkonen P, Boes T, Floss T, Schroeder J, Dearie F, Crunkhorn S, Burak F, Jimenez-Chillaron JC, Kuulasmaa T, Miettinen P, Park PJ, Nasser I, Zhao Z, Zhang Z, Xu Y, Wurst W, Ren H, Morris AJ, Stamm S, Goldfine AB, Laakso M, Patti ME. Expression of the splicing factor gene SFRS10 is reduced in human obesity and contributes to enhanced lipogenesis. *Cell Metab*. 2011; 14:208–218. [PubMed: 21803291]

Highlights

- Transformer2 (tra2) is a splicing protein whose metabolic functions are unknown.
- Tra2 functions in the *Drosophila* fat body to modulate triglyceride levels.
- Decreasing tra2 in the fat body promotes starvation resistance.
- Tra2 alters CPT1 splicing in the fly fat body.

Figure 1.

Decreasing *tra2* levels in the *Drosophila* fat body results in obese flies and enhanced starvation resistance. **(A)** *tra2* mRNA levels were measured by qPCR in adult, female yolk-Gal4>tra2^{dsRNA} fat bodies and compared to yolk-Gal4>atp2 controls. Bars represent mean and error bars represent standard error. * $p < 0.05$ using Student's t-test. **(B)** 1-week old yolk-Gal4>tra2^{dsRNA} female flies have an obese phenotype with an enlarged abdomen compared to yolk-Gal4>atp2 controls. **(C)** Female yolk-Gal4>tra2^{dsRNA} and yolk-Gal4>atp2 adult flies were put on starvation media and the number of dead flies was measured every 6 hours. Survival curves were analyzed using the Kaplan-Meier Estimator on OASIS. $p < 0.05$ using

Log Rank test when comparing mean lifespan and Fisher's Exact test when comparing 50% survival on starvation medium of both groups of flies.

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Figure 2.

Decreasing *tra2* in the fly fat body results in increased triglyceride storage. (A) Triglyceride and (B) glycogen levels were measured and normalized by total protein content for yolk-Gal4>tra2^{dsRNA} flies and compared to yolk-Gal4>atp2 controls. Bars represent mean and error bars represent standard error. *p<0.05 using Student's t-test.

Figure 3.

Decreasing fat body *tra2* results in fewer fat body cells and more fat per cell. **(A)** DNA and **(B)** triglyceride levels were measured in yolk-Gal4>tra2^{dsRNA} fat bodies and compared to yolk-Gal4>atp2 controls. In panel B, total triglycerides were normalized by DNA content to represent the amount of fat per cell. Bars represent mean and error bars represent standard error. *p<0.05 using Student's t-test.

Figure 4.

CPT1 splicing is altered in fat bodies with decreased *tra2*. **(A)** Food consumption was monitored over a 24-hour period using the CAFÉ assay in adult female yolk-Gal4>tra2^{dsRNA} flies and compared to yolk-Gal4>atp2 controls. **(B)** Diagram of 6A and 6B-including splice variants of *Drosophila* CPT1 (adapted from [8]). **(C)** qPCR was performed to amplify CPT1 isoforms containing exon 6A and 6B. The percent of transcripts including exons 6A and 6B of CPT1 in yolk-Gal4>atp2 and yolk-Gal4>tra2^{dsRNA} flies is shown above. Bars represent mean and error bars represent standard error. *p<0.05 using Student's t-test comparing

percent inclusion of splice variant 6A and 6B in each genotype. Mikoluk et al., “The splicing factor transformer2 (tra2) functions in the *Drosophila* fat body to regulate lipid storage”

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