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The role of larval fat cells in adult *Drosophila melanogaster*

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Summary

In the life history of holometabolous insects, distinct developmental stages are tightly linked to feeding and non-feeding periods. The larval stage is characterized by extensive feeding, which supports the rapid growth of the animal and allows accumulation of energy stores, primarily in the larval fat body. In *Drosophila melanogaster* access to these stores during pupal development is possible because the larval fat body is preserved in the pupa as individual fat cells. These larval fat cells are refractive to autophagic cell death that removes most of the larval cells during metamorphosis. The larval fat cells are thought to persist into the adult stage and thus might also have a nutritional role in the young adult. We used cell markers to demonstrate that the fat cells in the young adult are in fact dissociated larval fat body cells, and we present evidence that these cells are eventually removed in the adult by a caspase cascade that leads to cell death. By genetically manipulating the lifespan of the larval fat cells, we demonstrate that these cells are nutritionally important during the early, non-feeding stage of adulthood. We experimentally blocked cell death of larval fat cells using the GAL4/UAS system and found that in newly eclosed adults starvation resistance increased from 58 h to 72 h. Starvation survival was highly correlated with the number of remaining larval fat cells. We discuss the implications of these results in terms of the overall nutritional status of the larva as an important factor in adult survival in environmental stresses such as starvation.

Key words: cell death, fat body, starvation resistance, autophagy.

Introduction

The complex life cycle of holometabolous insects involves morphologically and ecologically distinct larval and adult stages, separated by the non-feeding pupal stage. In the case of *Drosophila melanogaster* (Meigen 1830), the last three days of larval development are characterized by a 200-fold increase in mass (Church and Robertson, 1966) and accumulation of nutrient reserves primarily in the larval fat body, a single-cell thick tissue composed of fat cells. These larval fat cells serve as an energy reservoir to support the animal through the subsequent non-feeding period. An important but somewhat overlooked aspect of *Drosophila* development is that this non-feeding period includes a period of time both before and after metamorphosis. Prior to metamorphosis, the larva ceases feeding and 'wanders' for 12–24 h in search of a pupation site (Riddiford, 1993). After eclosion, the newly emerged adult remains inactive for approximately 8 h until the wings expand and the cuticle tans (Chiang, 1963) (J.R.A. and D.K.H., unpublished data). Larvae must therefore acquire enough nutrients not only to fuel developmental reorganization but also to survive the late larval and early adult periods. In nature, the new adult may also need to seek a new food source, if the fruit or other substrate upon which it developed is no longer available. Thus, sufficient larval-derived nutrients must be stored and remain available for use by the adult.

The unusual developmental history of the larval fat body complicates our understanding of its role as an energy reservoir and its effects on the overall physiology of the animal. During metamorphosis, most larval tissues undergo autophagy and cell death, whereas the adult progenitor cells, i.e. imaginal discs and histoblasts, undergo cell proliferation, differentiation and organogenesis to give rise to the adult structures (Bainbridge and Bownes, 1981; Bodenstein, 1950; Robertson, 1936). The fat body, however, is refractive to cell death, but does undergo an unusual transformation from an organized tissue to a loose association of individual fat cells (Hoshizaki, 2005; Nelliot et al., 2006). The phenomenon of fat-body tissue dissociation has been documented in Diptera (*D. melanogaster* and *Sarcophaga peregrina*) and Lepidoptera (*Calpodes ethlius*) and is likely to be a common feature of holometabolous insects (reviewed by Hoshizaki, 2005).

In *D. melanogaster*, the individual cells of the larval fat body persist throughout metamorphosis as freely floating fat cells dispersed throughout the body cavity of the pupa (Butterworth, 1972; Hoshizaki, 2005; Nelliot et al., 2006). The newly eclosed adult contains freely floating fat cells that are likely to be

larval-derived fat cells. These cells later undergo cell death and are replaced by sheets of fat cells recognized as the adult fat body. The adult fat cells are most likely derived from cells embedded within the larval body wall and from adepithelial cells associated with imaginal discs (Hoshizaki et al., 1995). Fully differentiated adult fat cells are not easily recognized within the abdomen of the adult until 3–4 days post-eclosion. Although the adult fat cells are derived from a distinct and separate cell lineage from the larval fat body, both tissues share an important energy storage function.

Our focus in this study is the role of larval energy stores in the adult fly. Using cell markers we have identified the free-floating fat cells in the young adult as larval fat cells and experimentally extended their lifespan. We hypothesized that larval fat cells function in the young adult as 'meals-ready-to-eat' until the animal is flight-ready and successfully feeds. To test this hypothesis, we compared the ability of adults to resist starvation in the absence or presence of larval fat cells. Young adults harboring larval fat cells are nearly three times as resistant to starvation as older adults. The half-life of the larval fat cells is 9 h, and unfed adults begin to die from starvation once 85% of the larval fat cells have undergone cytolysis. We experimentally manipulated the lifespan of the larval fat cells and found that unfed adults are more starvation resistant when death of these cells is blocked. These data suggest that nutrients acquired by the larva and stored within the larval fat cells can contribute to adult stress resistance. Thus, larval fat cells have a fundamental role in post-metamorphic energy metabolism and provide an effective energy reserve important to the young adult animal.

Materials and methods

Drosophila husbandry and genetic crosses

All flies were raised at 25°C on a corn meal-soy flour-molasses-corn syrup medium (corn meal 80 g l^{-1}, molasses and corn syrup 36.3 ml l^{-1} each, yeast 18 g l^{-1}, soy flour 11 g l^{-1}, ethanol 12 ml l^{-1}, agar 6 g l^{-1}, propionic acid 5.2 ml l^{-1} and niapagen 1.2 g l^{-1}) supplemented with dry yeast.

The stocks (a) *y w; P{w[+mC]=UAS-n-syb.eGFP}3*, (b) *y w; P{Lsp2-GAL4.H}*, (c) *w; P{w[+mC]=UAS-p35.H}BH2*, (d) *w; P{w[+mC]=UAS-diap1.H}3* and (e) *w; P{w[+mC]=UAS-diap1.H}1* were obtained from the Bloomington Stock Center (Bloomington, IN, USA). The protein trap line G000343 was identified as part of a screen for proteins expressed in the larval fat body and salivary glands (Andres et al., 2004; Morin et al., 2001) and was generously provided by L. Cooley (Yale University, New Haven, CT, USA). The artificial exon encoding green fluorescent protein (GFP) in G000343 is inserted in-frame with a gene coding for a larval protein localized to polytene chromosomes (Andres et al., 2004) and is within chickadee but on the opposite strand, i.e. in the opposing reading frame (L. Cooley, unpublished data).

In separate experiments, we used the GAL4/UAS system of Brand and Perrimon to restrict expression of GFP to larval fat body cells (Brand and Perrimon, 1993). Briefly, the GAL4/UAS system is a bipartite system composed of a *GAL4* driver (*GAL4* transgene) and a *UAS* responder gene (*UAS* transgene). The *GAL4* driver in this case is *Lsp2-GAL4* (*P{Lsp2-GAL4.H}3*), a chimeric transgene composed of the promoter from the *larval serum protein 2* (*Lsp2*) gene and the coding sequence of the yeast *Saccharomyces cerevisiae GAL4* gene (C. Antoniewski, unpublished data).

Because the *Lsp2-GAL4* transgene contains the *Lsp2* promoter, it recapitulates the expression pattern of the endogenous *Lsp2* gene, which is expressed solely in larval fat body cells beginning early in the third larval instar (B. Hassad, personal communication to FlyBase). Thus, Gal4 protein encoded by *Lsp2-GAL4* is produced only in the larval fat body cells in the identical temporal and spatial pattern of the endogenous LSP2 protein. Gal4 is a DNA-binding protein that recognizes a 17-basepair sequence that functions as an upstream activation sequence designated UAS. Binding of Gal4 protein to the UAS sequence is sufficient to activate transcription of a downstream gene. Thus, in animals carrying both *Lsp2-GAL4* and a chimeric gene containing a *UAS* promoter region fused to the coding sequence for GFP, i.e. *UAS-GFP*, (*P{w[+mC]=UAS-n-syb.eGFP}3*), the expression of the *GFP* gene occurs strictly in the larval fat body cells.

Standard genetic crosses were performed to recombine *UAS-GFP*, which serves as a cell marker, and the larval fat-cell driver transgene *Lsp2-GAL4* onto the same chromosome. The final stock is homozygous for the genotype *y w; P{Lsp2-GAL4.H}, P{w+mc=UAS-n-syb.eGFP}3* and is abbreviated as *Lsp2-GAL4::UAS-GFP*. This stock specifically marks the larval fat body cell with GFP and is used in conjunction with other UAS transgenes to target expression to this tissue.

Two different cell death inhibitor genes, *p35* and *Drosophila inhibitor of apoptosis 1* (*diap1*), were employed to block cell death in the larval fat cells. Ectopic expression of *p35* or *diap1* was achieved using the GAL4/UAS system (Brand and Perrimon, 1993). Individuals carrying a UAS transgene for either *p35* or *diap1*, i.e. *UAS-p35* (*P{w[+mC]=UAS-p35.H}BH2*) or *UAS-diap1* (either *P{w[+mC]=P{UAS-DIAP1.H}3* or *P{w[+mC]={UAS-DIAP1.H}1*), were crossed with *Lsp2-GAL4::UAS-GFP* to drive ectopic expression of either *p35* or *diap1* to the larval fat cells and thus block cell death in these cells.

Quantitative analysis of larval fat cells

Two methods were used to quantify the number of larval fat cells in the adult. In the first method the abdomens of *Lsp2-GAL4::UAS-GFP* females were gently teased open and the free-floating larval fat cells were released into 1× Dulbecco's phosphate buffered saline (DPBS) (52 mmol l^{-1} NaCl; 40 mmol l^{-1} KCl; 10 mmol l^{-1} Hepes; 1.2 mmol l^{-1} $MgSO_4$; 1.2 mmol l^{-1} $MgCl_2$; 2 mmol l^{-1} Na_2HPO_4; 0.4 mmol l^{-1} KH_2PO_4; 1 mmol l^{-1} $CaCl_2$; 45 mmol l^{-1} sucrose; 5 mmol l^{-1} glucose, pH 7.2) on a 25×75 mm glass slide. Cells were examined by light and fluorescence microscopy to confirm that all larval fat cells expressed the GFP cell marker. A micro-grid

and a counter were used to physically count the number of larval fat cells in the abdomen.

In the second method, larval fat cells were quantified by GFP fluorescence. Intact *Lsp2-GAL4::UAS-GFP* aged females were mounted dorsal-side down onto 25×75 mm glass slides using GelMount (Sigma, St Louis, MO, USA). GFP fluorescence was measured using a Typhoon 8600 Variable Mode Imager and the intensity of the phosphoimage (in pixels) quantified using ImageQuant software.

Starvation resistance

For each genotype, newly eclosed females were collected immediately upon eclosion (0–10 min) and further identified by their deflated wings that have the appearance of flattened raisins. These adults were immediately assayed for starvation resistance or placed on food supplemented with yeast until tested. For starvation experiments, flies were divided into groups of 10 and starved in 47 mm plastic Petri dishes containing a disc of Whatman #42 ashless filter paper soaked with 650 μl of deionized water. Flies were maintained at 25°C, and mortality rates were determined by counting the number of dead flies every three hours. The starvation graphs are the average percent survival for *N* groups of 10 animals over time and error bars represent standard deviations.

Fluorescent and confocal imaging

Fluorescent and confocal microscopy was performed in the Nevada INBRE Center for Biological Imaging using a Zeiss LSM-510 microscope and LSM-510 Axioplan 2 Imaging software. Freely floating fat-body cells were obtained from *Lsp2-GAL4::UAS-GFP* females and mounted in 1× DPBS. Cells were analyzed within an hour after slide preparation.

Results

Adults flies starved upon eclosion are more resistant to starvation than older flies

We hypothesized that the free-floating fat cells found in the newly eclosed adult represent an important energy reserve. Because these cells are absent in 3-day-old adults, we initially tested our hypothesis by comparing the starvation resistance of newly eclosed adults carrying mutations *yellow* (*y*) and white (*w*) with older *y w* adults. Groups of 10 *y w* females were collected upon eclosion (0–10 min) and either immediately tested for starvation resistance or aged on food supplemented with yeast before testing. We found that newly eclosed female adults were more resistant to starvation (LD_{50}=45 h) than 3- or 10-day-old animals (LD_{50}=16 h and 14 h, respectively; Fig. 1). These data support the idea that the free-floating fat cells represent a significant energy source.

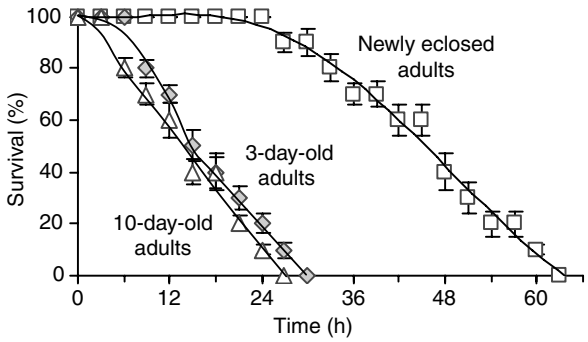

Fig. 1. Starvation resistance of *y w* adult flies decreases with age. Starvation resistance was measured by percentage survival of adult females in groups of 10 flies. Newly eclosed *y w* adults (*N*=20 groups of 10) (squares), 3-day-old *y w* adults (*N*=30 groups of 10) (diamonds), 10-day-old *y w* adults (*N*=10 groups of 10) (triangles). Values are means ± s.d.

Freely floating fat cells in the adult are the larval fat cells

During metamorphosis the larval fat-body dissociates to give rise to individual fat cells that persist throughout pupal development. It is commonly accepted that the freely floating fat cells in the adult are the cells from the dissociated larval fat body (Butterworth, 1972; Hoshizaki, 2005; Nelliot et al., 2006). We re-examined the origin of the freely floating fat cells in the adult because it is important to our understanding of the energy flow that supports the young adult and in defining the underlying basis of the higher starvation resistance of newly eclosed adults.

To experimentally establish the origin of these cells in the young adult, we took advantage of a GFP protein trap line for a polytene chromosome-associated protein (Andres et al., 2004). Polytene chromosomes are a hallmark of larval tissues including the fat body. We used this cell marker to distinguish between adult tissues that contain mitotic chromosomes and larval polytenized tissues. As expected, the free-floating fat cells in the newly eclosed adult were GFP-labeled, thus confirming their larval origin (Fig. 2).

To begin to understand the contribution of the larval fat cells to the young adult, we developed a GAL4/UAS GFP-based assay to monitor the presence of these cells in the adult. We used a homozygous transgenic line, *Lsp2-GAL4::UAS-GFP*, in which GFP is expressed only in the larval fat body (Nelliot et al., 2006). Thus, in the adult the only GFP-positive cells are the fat cells from the dissociated larval fat body. We determined the rate at which these cells were lost in the adult by following the loss of GFP fluorescence by measuring phosphoimage intensity (Fig. 3). GFP fluorescence was quantified for individual aged female adults and compared with the number of larval fat cells obtained by dissection of individual animals (Fig. 4); GFP fluorescence was proportional to the number of larval fat cells. Thus, by measuring GFP fluorescence we can monitor the transient presence of larval fat cells in the adult. We found that within ~9 h post-eclosion, 50% of the larval fat cells have undergone cytolysis (Fig. 4, and see Fig. 6).

Transgenic adults starved upon eclosion were also more resistant to starvation than older adults

We next tested whether starvation accelerates the rate of cytolysis of the larval fat cells, thereby allowing a more rapid

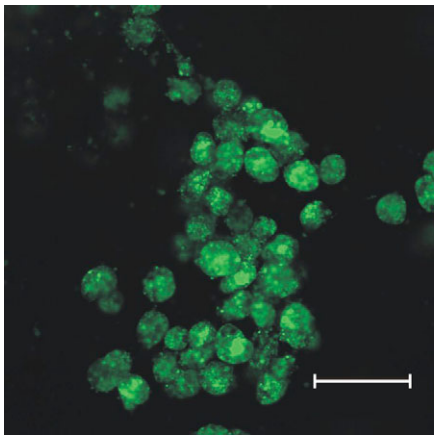

Fig. 2. Free-floating fat cells in the adult are dissociated larval fat body cells. Free-floating fat cells from an adult labeled with a polytene chromosome GFP cell marker (*G000343/CyO*). Scale bar, 200 μm.

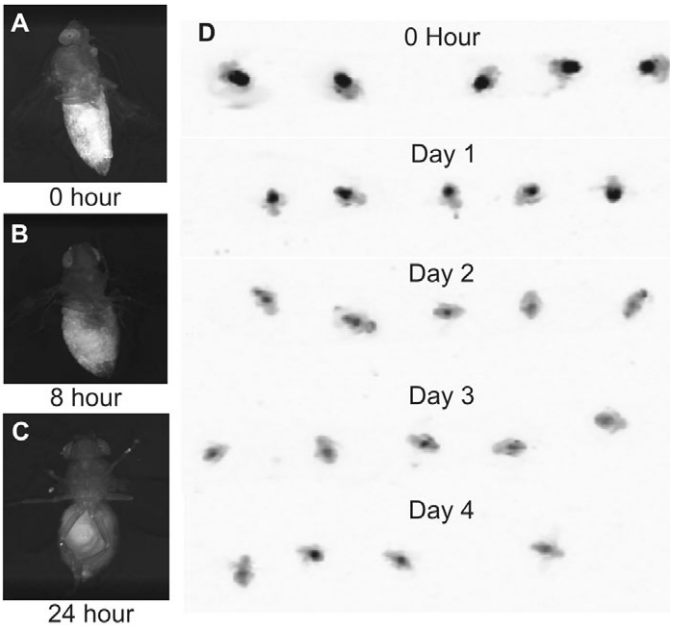

Fig. 3. Whole-mount adults used for GFP-based measurement of larval fat cells. (A–C) Fluorescent images of whole-mount *Lsp2-Gal4::UAS-GFP* aged adult females. GFP-labeled larval fat cells are prominent in the abdomen. (D) Phosphoimage of whole-mount *Lsp2-Gal4::UAS-GFP* aged adult females used to quantify larval fat cells.

recycling of bulk nutrients. This increase in nutrient recycling might be a mechanism contributing to starvation resistance. As a control we first tested whether the presence of the *Lsp-GAL4::UAS-GFP* transgenes affected starvation resistance. We found that the presence of the transgenes had no effect on starvation resistance; newly eclosed animals were still more resistant (LD_{50}=58 h) than 3- or 10-day-old adults (LD_{50}=26 h and 20 h, respectively; Fig. 5). To test the effects of starvation, we monitored the loss of fat cells using the GFP-based assay in newly eclosed *Lsp-GAL4::UAS-GFP* animals. Surprisingly,

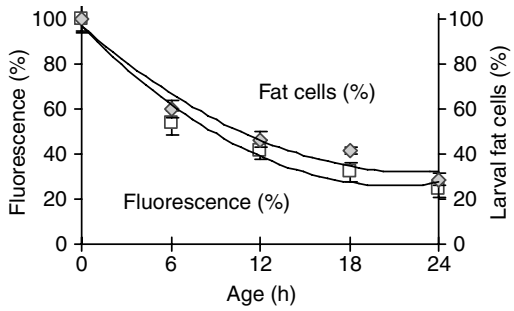

Fig. 4. GFP fluorescence in adults is directly related to *in situ* percentage fat-cell number. Larval fat-cell number for *Lsp2-Gal4::UAS-GFP* adult females using the GFP-based assay (*N*=44–60 individuals per time point) compared with *in situ* fat-cell numbers from dissected individual females (*N*=28–46 individuals per time point). Values are means ± s.d. Squares, percentage fluorescence; diamonds, percentage cell number.

starvation did not affect the rate of larval fat-cell cytolysis; within 8.5 h post-eclosion, 50% of the fat cells had undergone cell death (data not shown). We note that adults began to succumb to starvation when approximately 85% of the larval fat cells were lost (Fig. 6). These data suggest that larval fat cells represent a significant energy reserve and that mobilization of fat-cell energy stores is not solely dependent upon bulk recycling of fat-cell components released upon cell death.

Larval fat cells increase starvation resistance in the adult

To directly test whether larval fat cells contribute to adult starvation resistance, we inhibited the normal cell death of the larval fat cells. We employed both the *Drosophila* inhibitor of apoptosis 1 (DIAP1) protein and the baculovirus p35 protein, both of which directly inhibit the caspase cascade leading to apoptotic cell death (Wang et al., 1999; Wilson et al., 2002). Ectopic expression of either *p35* or *diap1* in the larval fat cells was accomplished using the larval fat-cell driver *Lsp2-Gal4* (i.e. *Lsp2-GAL4::UAS-GFP)* and either the *UAS-p35* or *UAS-diap1* transgene. As a control we tested whether the inhibition of cell death in the fat body affects the total number of fat cells. We compared the number of larval fat cells present in the newly eclosed control adults (*Lsp2-GAL4::UAS-GFP*) with the number of larval fat cells in the experimental adults (*Lsp2-GAL4::UAS-GFP + UAS-diap1*) (Fig. 7); we found that an equal number of fat cells was present.

The newly eclosed experimental animals (either *Lsp2-GAL4::UAS-GFP + UAS-p35* or *Lsp2-GAL4::UAS-GFP + UAS-diap1*) were then tested for starvation resistance; these animals exhibited increased starvation resistance from LD_{50}=57 h to LD_{50}=82 h (Fig. 8). To determine whether the increase in starvation resistance was correlated with an extended lifespan of the larval fat cells, we physically counted the number of larval fat cells in *Lsp2-GAL4::UAS-GFP + UAS-diap1* animals (Fig. 7A). At 24 h post-eclosion, when ~70% of the fat cells have normally undergone cytolysis, only 38% of

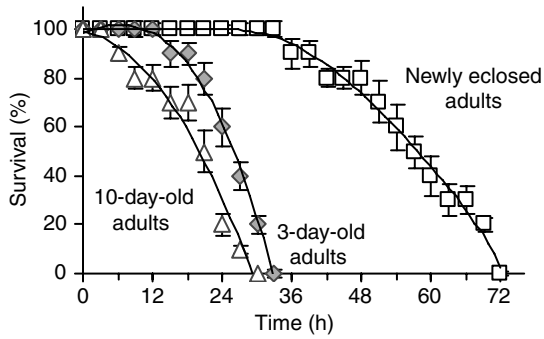

Fig. 5. Starvation resistance of *Lsp2-Gal4::UAS-GFP* adults decreases with age. Starvation resistance was measured by percentage survival of adult females in groups of 10 flies. Newly eclosed *Lsp2-Gal4::UAS-GFP* adults (*N*=8 groups of 10) (squares), 3-day-old *Lsp2-Gal4::UAS-GFP* adults (*N*=14 groups of 10) (diamonds), 10-day-old *Lsp2-Gal4::UAS-GFP* adults (*N*=10 groups of 10) (triangles). Values are means ± s.d.

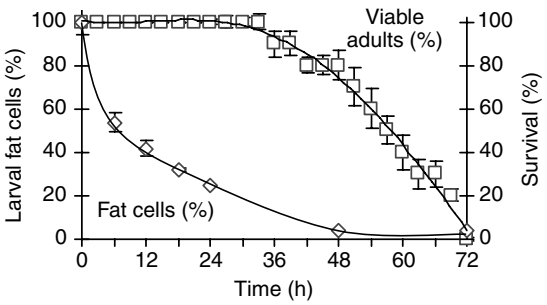

Fig. 6. Larval fat-cell number and starvation resistance in newly eclosed adults. Larval fat-cell number measured for *Lsp2-Gal4::UAS-GFP* adult females using the GFP-based assay (*N*=25–36 per time point), and compared with the percentage survival of newly eclosed *Lsp2-Gal4::UAS-GFP* starved adult females (*N*=8 groups of 10). Diamonds, percentage fluorescence; squares, percentage survival. Values are means ± s.d.

fat cells were absent in the adults in which cell death was blocked. The increased survivorship of fat cells in the experimental adults was also detected at 48 h, when cytolysis of the larval fat cells is normally complete. In the cell death-blocked animals, 40% of the fat cells were still present. Finally, at 72 h experimental adults began to succumb to starvation while ~22% of the larval fat cells remained (compare Fig. 7A with Fig. 8).

Discussion

The life cycle of *D. melanogaster* is characterized by feeding and non-feeding periods that are linked to specific developmental stages. During the larval stage energy reserves are acquired and stored in the larval fat body to be used to fuel the re-architecture of the animal to the adult form during metamorphosis. The underlying mechanisms controlling mobilization of energy stores from the fat cells during metamorphosis are not known, although it has been suggested that autophagy plays a fundamental role in this process (Rusten et al., 2004). Most larval tissues undergo autophagy leading to cell death, thereby allowing bulk recycling of components; however, the fat body undergoes tissue remodeling leading to the dissociation of the fat body (Nelliot et al., 2006). In addition to supporting pupal development, sufficient larval energy stores must also be in reserve to support the newly eclosed adult until a suitable foraging site is located. We present here the first experimental evidence that the energy reservoirs acquired during the larva feeding period are carried into the adult by free-floating cells derived from the dissociated fat body. By employing GFP cell markers, we demonstrated that the free-floating fat cells are larval in origin and have established a profile measuring the loss of these cells in the young adult. Correlated with the loss of larval fat cells is an increased sensitivity to starvation. By genetic manipulation, we have inhibited cell death of the larval fat cells in the adult and have correspondingly increased starvation resistance. These data demonstrate that the larval fat cells serve as ‘meals-ready-to-eat’ for young adults and are of importance for individuals that have developed on ephemeral breeding sites and which must relocate to new feeding sites.

Larval fat cells in the adult

Through the use of cell markers, we have demonstrated that the free-floating fat cells in the adult are the dissociated cells from the larval fat body (Fig. 2). We have determined the number of free-floating fat cells in the abdomen of the newly eclosed female adults to be 766 (*N*=49; s.d.=49), which is in contrast to the 1052 cells (*N*=8; s.d.=177) estimated by Butterworth (Butterworth, 1972). We believe the discrepancy between our results and those of Butterworth lies in our improved ability to identify larval fat cells. In our *in situ* counts, the fat cells express GFP, thereby allowing easy identification of the cells from other free-floating cells and debris. By contrast, Butterworth examined unstained samples and, as noted by Butterworth (Butterworth, 1972), the *in situ* counts are likely to include cells from other tissues.

It has been estimated that the female larval fat body is made up of 2500 fat cells (Rizki, 1969). After tissue dissociation during metamorphosis, 20% of the fat cells are thought to reside in the pupal head, with some cells in the thorax (Rizki, 1969). Based on these estimations, approximately 2000 fat cells should be present in the abdominal region of the pupa. In newly eclosed adults, however, far fewer fat cells were recovered (Butterworth, 1972) (this study). This discrepancy might reflect partial elimination of larval fat cells during pupal development (Butterworth, 1972), or the estimated distribution of fat cells in the pupa might not be correct. Our recent descriptive analysis of fat-cell dissociation in the early pupa indicates that a substantial proportion of the fat cells reside in the thorax [fig. 1 in Nelliot et al. (Nelliot et al., 2006)]. We estimate that in the early stage pupa, at least half of the fat cells reside in the pupal head and thorax. Therefore, the pupal abdomen should contain

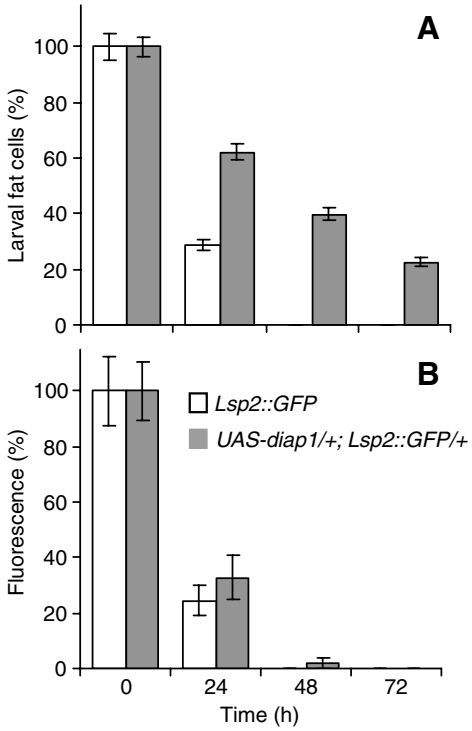

Fig. 7. Larval fat cells persist in aged adults when cell death is blocked. (A) *In situ* fat-cell number from *Lsp2-Gal4::UAS-GFP/UAS-diap1* adult females in which cell death is blocked (*N*=15–20 individuals per time point, filled bar) compared with *Lsp2-Gal4::UAS-GFP* control adult females (*N*=10–46 individuals per time point, open bar). (Mean initial cell number for *Lsp2-Gal4::UAS-GFP/UAS-diap1* was 792 cells; for *Lsp2-Gal4::UAS-GFP* it was 724 cells.) (B) GFP-fluorescence of *Lsp2-Gal4::UAS-GFP/UAS-diap1* adult females (*N*=15–25 per time point, filled bar). *Lsp2-Gal4::UAS-GFP* control adult females (*N*=10–60 individuals per time point, open bar). (Mean initial fluorescence for *Lsp2-Gal4::UAS-GFP/UAS-diap1* was 25 600 pixels; for *Lsp2-Gal4::UAS-GFP* it was 21 900 pixels.) Note, perdurance of GFP-fluorescence does not reflect fat-cell number in the cell death-blocked animals. This is probably because of a loss of activity from the *Lsp2* promoter (see Discussion for details). Values are means ± s.d.

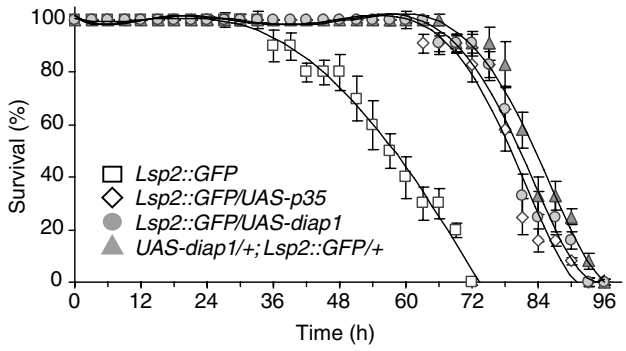

Fig. 8. Starvation resistance increases in adults carrying larval fat cells in which cell death is blocked. Starvation resistance was measured by percentage survival of newly eclosed adult females in groups of 10 individuals. Control, *Lsp2-Gal4::UAS-GFP* adults (*N*=80 groups of 10, squares). Larval fat cells with extended lifespan, *Lsp2-Gal4::UAS-GFP/UAS-p35* adults (*N*=20 groups of 10, diamonds), *Lsp2-Gal4::UAS-GFP/UAS-diap1* adults (*N*=20 groups of 10, circles), and *UAS-diap1/+*; *Lsp2-Gal4::UAS-GFP/+* adults (*N*=20 groups of 10, triangles). Values are means ± s.d.

approximately 1250 cells. Our mean number of cells recovered from the adult abdomen was 766, only 60% of the predicted number of cells.

It is possible that a portion of the fat cells undergo cell death during pupal development, but we believe this to be unlikely for two reasons. First, we have measured the number of fat cells at the beginning of pupal development using the GFP-assay and find that this number remains the same between white prepupae and newly eclosed adults (data not shown). Second, the inhibition of apoptotic cell death by expression of *diap* or *p35* did not change the number of fat cells recovered in the newly eclosed adult. These data indicate that few larval fat cells are eliminated during pupal development. The discrepancy in the predicted cell number in the adult abdomen might be due in part to the incomplete efficiency in recovering the abdominal fat cells for *in situ* counts and/or distribution of fat cells in the early pupa might be altered during later pupal development.

Mechanism of larval fat cell cytolysis in the adult

During metamorphosis the fat body is refractive to cell death and does not begin to undergo cytolysis until after eclosion. Based on our measurements, cytolysis is essentially complete by 48 h of adult development (Fig. 6). The factors that control or trigger fat-cell cytolysis and the underlying mechanism by which cell death is achieved are not known. It has been suggested that juvenile hormone and the gene *apterous* might participate in triggering programmed cell death in the fat cells (Butterworth, 1972; Postlethwait and Jones, 1978), but a reassessment of the *apterous* mutant (Richard et al., 1993) suggests otherwise (reviewed by Hoshizaki, 2005). We suggest that the cytolysis signal is also not likely to be a nutritional cue because we did not observe an accelerated rate of larval fat cell loss in starved adults.

We note that in adults in which fat-cell death is blocked, expression of GFP in the fat cells does not correspond to the *in situ* number of fat cells (Fig. 7). We surmise that the ectopic activity of the *Lsp2-GAL4* is compromised in the adult and does not allow for maintenance of GFP beyond 48 h. Under normal conditions, this is not a concern for the GFP-based assay because removal of fat cells is complete by this time. If the activity of *Lsp2-GAL4* is compromised, then it follows that the expression of the *UAS-diap1* would also be compromised. If induction of cell death occurs immediately after eclosion, then expression of cell death inhibitors, such as *diap1* and *p35*, during this window should be sufficient to prevent loss of fat cells. The nature of subsequent removal of the remaining larval fat cells at 72 to 96 h post-eclosion is not known and is currently under investigation.

The programmed cell death of the larval fat cells is the final

and normal step in the developmental history of this tissue. Two major classes of programmed cell death, type 1 (apoptotic) and type 2 (autophagic), are recognized as normal processes for remodeling tissues, controlling cell number and eliminating abnormally damaged cells. Apoptotic cell death is characterized by cellular and nuclear shrinking, association of chromatin with the nuclear periphery, DNA fragmentation, formation of apoptotic bodies, caspase activation and the engulfment and lysosomal degradation of the dying cell by a phagocyte (Kerr et al., 1972). Autophagic cell death, however, is a membrane trafficking process involving autophagosomes that engulf cytosol and organelles and then are fused with lysosomes to form autolysosomes in which the cargo undergoes hydrolysis (Yoshimori, 2004).

The major signal that triggers metamorphosis and larval tissue histolysis is the high titer pulse of ecdysone that occurs at puparium formation, i.e. the larval-pupal transition. Most larval tissues undergo histolysis, with the notable exception of the fat body, which is remodeled from an intact tissue to detached cells (Nelliot et al., 2006). Larval histolysis is associated with formation of acidic autophagic vesicles consistent with an autophagic cell death response. However, histolysis is also accompanied by hallmarks of apoptosis. The degenerating prothoracic and labial glands of the tobacco horn worm *Manduca sexta*, for example, are accompanied by highly condensed chromatin indicative of apoptosis (Dai and Gilbert, 1997; Jochova et al., 1997), whereas the *D. melanogaster* salivary glands are characterized by DNA fragmentation (Jiang et al., 1997). Furthermore, inhibition of caspase activity by p35 blocks DNA fragmentation and salivary gland cell death (Jiang et al., 1997; Lee and Baehrecke, 2001) and expression of *diap1* (a direct inhibitor of caspase activity) in the salivary glands is required throughout larval development to inhibit *reaper*- and *head involution defective*-triggered apoptotic cell death (Yin and Thummel, 2004). Based on these observations, we surmise that larval tissue histolysis might be accompanied by autophagy to allow efficient recycling of larval cellular components during metamorphosis and in the young adult, and that the final destruction of the cell in the aged adult is dependent upon apoptotic cell death.

A developmental conundrum, however, is presented by the larval fat body. Ecdysone signaling that triggers histolysis in most larval tissue triggers fat-cell dissociation but not cell death, which is delayed until adult stage. The final destruction of the fat cells, however, is also inhibited by expression of *diap1* and *p35*, thereby suggesting that fat cell death is through a process similar to that used to remove the other larval tissues. Further studies are needed to understand why the fat body is initially refractive to cell death while other larval tissues are destroyed, and the relationship between apoptotic cell death and recycling of cellular components (macroautophagy) in larval fat cells of the adult.

Importance of larval energy stores for adult performance

The natural feeding and oviposition site of *D. melanogaster*, rotting fruit, is an ephemeral resource. Eclosing flies may have no food available, but their ultimate evolutionary success depends upon finding a foraging and breeding site that leads to successful reproduction. The larval fat cells may therefore contribute to the success of the adult by serving as a reserve energy source in case foraging is delayed (e.g. by the deterioration of the pupal development site or by inclement weather). It is also important to note that energy expenditure during pupation and early adulthood will vary according to temperature. *Drosophila* habitats can vary widely in temperature, on timescales of minutes to days (Feder et al., 1997; Gibbs et al., 2003), so a reserve of larval-derived energy may prove essential for adult success.

Although larval-derived energy may be essential for the success of individual adults, selection experiments indicate there is a trade-off between energy storage and other life history parameters. Starvation-selected populations of *D. melanogaster* store more energy in the larval stage than unselected control populations, but they develop more slowly and their egg-to-adult viability is lower (Chippindale et al., 1996; Chippindale et al., 1998). Similar patterns can be found in desiccation-selected lines, in which larval accumulation of water and glycogen leads to slower development (Chippindale et al., 1998; Gefen et al., 2006).

At the organismal level, our most surprising finding is that starvation resistance decreased during the first 3 days of adult life, despite the fact that flies were able to feed and presumably store energy. Similar results have been obtained for several other *Drosophila* species (Sevenster and Vanalphen, 1993), although not all (Baldal et al., 2004). A likely explanation for this phenomenon is allocation of resources to reproduction during early adulthood. Once these resources are committed to gonad development they might not be readily available to the soma to support the animal during starvation. When *D. melanogaster* are provided with a high-protein diet, energy storage declines as fecundity and metabolic rates increase (Simmons and Bradley, 1997). Resources acquired during the first few days of adult life may be preferentially directed to reproduction, rather than stored as an energy reserve. This is in accordance with *D. melanogaster* being considered a ‘fast’ species (Sevenster and Vanalphen, 1993) that develops and breeds rapidly at the expense of adult survival.

Conclusion

Nutrient stores acquired by the larva are transferred to the adult in the dissociated cells of the larval fat body. These larval fat cells appear to be a very efficient source of nutrients compared with the adult fat cells, based on the observation that newly eclosed adults are nearly three times as resistant to starvation as older fed flies. The ability of newly eclosed adults to resist starvation, however, goes beyond their access to fat-cell energy stores left over from pupal development. By blocking cytolysis of the larval fat cells, starvation resistance can be further increased by more than 24 h. This increase is not because of an increase in the number of larval fat cells in the newly eclosed adult. One possible explanation is that energy

stores contained within the fat cells are more easily mobilized to support the starving animal than energy stores previously released by cell death or autophagy and distributed in other tissues or hemolymph. Thus, not all energy stores in the adult fly may be equally accessible.

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