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The role of larval fat cells in starvation resistance and reproduction in adult Drosophila melanogaster

Jerell Roland Aguila
University of Nevada, Las Vegas

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THE ROLE OF LARVAL FAT CELLS IN STARVATION RESISTANCE AND REPRODUCTION IN ADULT *DROSOPHILA MELANOGASTER*

by

Jerell Roland Aguila

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Jerell Roland Aguila

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[redacted]
Examination Committee Chair

[redacted]
Dean of the Graduate College

[redacted]
Examination Committee Member

[redacted]
Examination Committee Member

[redacted]
Examination Committee Member

[redacted]
Graduate College Faculty Representative

ABSTRACT

The Role of Larval Fat Cells in Starvation Resistance and Reproduction in Adult *Drosophila melanogaster*

by

Jerell Roland Aguila

Dr. Deborah K. Hoshizaki, Examination Committee Chair
Associate Professor of Life Sciences
University of Nevada, Las Vegas

The intricate life cycle of holometabolous insects includes well-defined larval and adult stages associated with feeding and non-feeding periods. The larval stage is distinguished by prevalent feeding and is necessary for supporting the animal as it quickly grows. The larval stage also serves as the period for the animal to obtain adequate energy stores, primarily in the larval fat body, to fuel the animal through the non-feeding pupal and immature adult stages. Acquiring sufficient energy stores is paramount for the success of the adult animal. In fact, certain insects, such as silkworms and mayflies, do not feed as adults and must obtain all their lifetime nutrients during the larval stage. In *Drosophila melanogaster*, the larval fat body is preserved during the pupal stage as individual dissociated cells, enabling the animal access to the energy stores. These larval fat cells do not undergo early pupal autophagic cell death that eliminates most of the larval cells during metamorphosis. Instead, these larval fat cells persist into the adult stage and have a nutritional role in the young adult. By utilizing cell markers, I show that the larval fat cells remain in the young adult and are ultimately removed in the adult by a

caspase cascade leading to cell death. In addition, I demonstrate that the larval fat body plays a key role in enhancing starvation resistance and serving as a nutritional reservoir for the adult animal. I also report here that the rapid release of energy stores from larval fat cells by caspase-induced cell death promotes the rapid maturation of the ovaries and has an important role in establishing female fecundity. Furthermore, I suggest that the transfer of larval nutrients from the larval fat body to the adult gonadal tissues is necessary for the proper development of the ovaries. Finally, I demonstrate that in the absence of caspase-induced programmed cell death, the age of first reproduction is delayed and total fecundity is reduced in females. Overall these results reveal an important role for the larval fat reserves in the rapid development of the ovaries, which I propose in a model at the end of the dissertation.

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LIST OF ABBREVIATIONS

^{12}C – carbon 12
^{13}C – carbon 13
C - celsius
$CaCl_2$ – calcium chloride
$\delta^{12}C$ – carbon 12 isotope ratio
$\delta^{13}C$ – carbon 13 isotope ratio
Dpp-1 – decapentaplegic 1
Diap1 – drosophila inhibitor of apoptosis 1
Diap2 – drosophila inhibitor of apoptosis 2
DNA – deoxyribonucleic acid
DPBS – Dulbecco's phosphate buffered saline
g – gram
GFP – green fluorescent protein
hid – head involution defect
IAP – inhibitors of apoptosis
K^+ - potassium
KCl – potassium chloride
KH_2PO_4 – potassium dihydrogen phosphate
l – liter
Lsp2 – larval serum protein 2 gene
Lsp2-GAL4::UAS-GFP - y w; P{Lsp2-GAL4.H}, P{w+mc=UAS-n-syb.eGFP}3
$MgCl_2$ – magnesium chloride
$MgSO_4$ – magnesium sulfate
ml – milliliter
mm – millimeter
mmol – millimolar
NaCl – sodium chloride
Na_2HPO_4 – sodium phosphate
pH – logarithm of the reciprocal of hydrogen-ion concentration in gram atoms per liter
PCD – programmed cell death
PDB – Peedee Belemnite
RHG motif – Reaper, Hid, Grim motif
RNA – ribonucleic acid
rpr – reaper gene
SD – standard deviation
TOR – target of rapamycin
UAS – upstream activation sequence
UAS-diap1- P{w[+mC]= {UAS-DIAP1.H}1
UAS-p35 - P{w[+mC]=UAS-p35.H}BH2

μL – microliter
μg - microgram
w – white gene
y – yellow gene

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CHAPTER 1

INTRODUCTION

Energy homeostasis is important for the fitness and survival of all organisms and entails energy acquisition, storage, and tissue specific allocation. In *Drosophila melanogaster,* the fat body plays a significant role in both intermediary metabolism and the storage of nutrients. It has also been shown to serve as a nutrient sensor that affects organismal growth and longevity (Colombani et al., 2003). Through insulin and TOR signaling, the fat body directly influences the growth of cells and the overall size of the organism (Hafen, 2004). In addition, previous studies have demonstrated a role for the fat body in the production of growth factors (Kawamura et al., 1999) and in the synthesis and secretion of antimicrobial defense peptides (Hoffmann et al., 1996). Finally, the larval fat body functions to accrue sufficient energy stores to fuel pupal development and to sustain the young adult until it begins foraging.

In *D. melanogaster,* metamorphosis is characterized by an extraordinary transformation from the larval to the adult form. The specialized larval imaginal cells proliferate to give rise to the adult tissues while the larval tissues are degraded through the process of autophagic programmed cell death (Lee and Baehrecke, 2001). A striking exception to this loss of larval tissues is the fat body, which undergoes tissue dissociation into individual cells (Nelliot et al., 2006). These larval-derived fat cells survive as independent cells throughout metamorphosis and are presumed to function as a

nutritional reservoir to fuel the re-architecture of the animal to the adult. The larval-derived fat cells persist and are present in the young adults, where they later undergo cell death.

In this dissertation, I present the first definitive evidence that energy stores acquired during larval development are transmitted to the adult *via* the larval fat cells. I hypothesize that the presence of larval fat cells in newly-eclosed females is instrumental in the three-fold increase in starvation resistance relative to older females (Aguila et al., 2007). Consistent with this hypothesis are my observations that the inhibition of programmed cell death in the larval fat cells results in a four-fold increase in starvation resistance (Aguila et al., 2007). While it has been assumed that larval fat cells serve as an important energy reservoir in the adult female, these data do not explain why females, where fat-cell energy stores are released by programmed cell death, are more sensitive to starvation than females in which death of the larval fat cells is delayed.

To explore this conundrum, I tested the role of larval fat cells as the source of energy stores transferred to the ovaries to support their proper development. I demonstrate that the transfer of energy stores from the larval fat cells to the ovaries is facilitated by programmed death of the larval fat cells. I also demonstrate that the inhibition of normal cell death of the larval fat cells leads to a decrease in the size and mass of adult ovaries, as well as a decrease in overall female fecundity. Overall, I address the importance of larval reserves in establishing female fecundity, the trade-off between growth and size, and life history traits.

Larval Diet Affects Adult Survival and Fecundity

In holometabolous insects, the acquisition of sufficient metabolic resources during the larval stages is paramount for the adult's success. The allocation of larval energy stores directly affects adult morphology, size control, and fitness. In fact, some insects do not feed as adults and must obtain all their nutrient reserves during the larval period. For example the silkworm, *Bombyx mori*, cannot fly in the adult phase and has reduced mouth parts making it unable to feed as an adult (Goldsmith et al., 2004). Likewise, adult mayflies have vestigial mouthparts and a digestive system filled with air, and thus do not feed. Their adult life-span ranges from only 30 minutes to one day, and their primary objective is to reproduce (McCafferty, 1994).

Some researchers have observed that the larval feeding period has a significant role in overall adult fitness. In *Manduca sexta*, it has been demonstrated that varying the amounts of sugar, protein, or water in the larval diet has a profound effect on adult eclosion time, size, and fat content (Raguso et al., 2007). Furthermore, in the butterfly *Speyeria mormonia*, adults with a smaller body mass and shorter forewing length resulted when their last larval instar food intake was reduced by half (Boggs and Freeman, 2005).

Recent work in *D. melanogaster* has also investigated the importance of the larval feeding period for adult success. *Drosophila* larvae restricted from feeding on yeast exhibit a delayed eclosion, small body size, reduced ovariole number, and reduced age-specific fecundity (Tu and Tatar, 2003). It has also been demonstrated that desiccation-selected *D. melanogaster* have a higher body mass resulting from a prolonged third instar larval feeding period (Gefen et al., 2006). Furthermore, flies that are selected for high

aggregated oviposition behavior are larger as adults compared to controls because of a significant increase in larval feeding rates (Ruiz-Dubreuil et al., 1996).

Larval Energy Stores: Importance for Life History Evolution

For any animal, limiting nutritional resources can lead to a metabolic tradeoff between growth, reproduction, and somatic maintenance. One important determinant of an animal's life history is the acquisition and allocation of nutrients (Rose and Bradley, 1998). In many insects, the acquisition and allocation of nutrients takes place at both the larval and adult stages. Limitations on nutrient usage from different life stages can be affected by changes in diet, anatomy, metabolism, and digestive physiology (Zera and Harshman, 2001). It is imperative for an insect to obtain sufficient energy stores at both the larval and adult stages in order to support growth, reproduction, and maintenance of the soma.

Energy resources in adults of holometabolous insects may originate either from larval or adult feeding. The Y model of resource allocation predicts a key tradeoff between reproduction and survival (Zera and Harshman, 2001). While it is clear that allocating energy stores to one will compromise the success of the other, the physiological mechanisms underlying the tradeoff are still largely unknown.

Since pupae do not feed, the energy required to fuel developmental changes during metamorphosis must be acquired and stored during the larval feeding period. In *D. melanogaster* the last three days of larval development are characterized by a 200-fold increase in mass (Church and Robertson, 1966). The accumulation of nutrient reserves is primarily in the larval fat body. Interestingly, during development there is a non-feeding period both before and after metamorphosis. Prior to metamorphosis, the larva stops

feeding and "wanders" for 12-24 hours in search of a pupation site (Riddiford, 1993). After eclosion, the newly-emerged adult remains inactive for approximately eight hours until the wings expand and the cuticle tans (Chiang, 1963). Larvae must therefore acquire enough nutrients not only to fuel developmental re-organization 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. Therefore, sufficient larval-derived nutrients must be stored and remain available for use by the adult.

Ovary Development in Drosophila melanogaster

The germ cell elements of the female reproductive system are derived from cells that are sequestered to the posterior pole of the embryo before blastoderm formation (Sonnenblick, 1950). These pole cells have RNA-rich polar granules within their cytoplasm and develop into a polar cap of 24 to 48 cells between the blastoderm and vitelline membrane (Sonnenblick, 1950). The pole cells destined to be incorporated into gonads move to the interior of the embryo within the lumen and form the posterior midgut rudiment. Later, they disperse from the gut lumen and move to the left or right side of the gut cells. These germ cells are then enveloped by a monolayer of cuboidal cells that originate from the somatic mesoderm. The mesoderm also supplies secondary cells that lie between the large germ cells (Poulson, 1950). A newly hatched larva contains minute ovaries that only have 8 to 12 oogonia, or precursor stem cells that occupy the cortex of the ovary and begin oogenesis (Sonnenblick, 1950).

Throughout the larval life of a female *Drosophila*, there are relatively little changes in the ovary apart from an increase in size. During larval development the ovary

will increase 50-fold in volume (Sonnenblick, 1941). For the duration of the larval and pupal periods, the ovaries only contain immature oogonia and no oocytes. About 36 hours after puparium formation, the ovaries become attached to the oviducts. The first oocytes appear much later in development, shortly before the emergence of the fly (Kerkis, 1933). Upon eclosion, the adult ovary is comprised of a cluster of parallel ovarioles containing egg chambers arranged in a single file. The ovaries of newly eclosed flies are not yet completely mature and contain no ripe eggs. The ovaries are made up of 15-20 egg tubes or ovarioles with the germarium as the anteriormost end. The exact number of ovarioles is dependent upon the larval food condition (Saveliev, 1928). The ovaries of 1-day-old flies contain eggs at various stages of development, and the amount of yolk deposited in each egg indicates its stage of development. Around two days after eclosion of the fly, the ovary contains fully developed eggs ready to be laid (Saveliev, 1928).

In *Drosophila melanogaster* females, the rate at which ovarioles complete development and the rate of egg lay can vary considerably based on several factors, including genotype, age, temperature, humidity, the abundance of mates, the quality of oviposition sites, and the overall nutrition of the animal (Robertson and Sang, 1944; Chiang and Hodson, 1950). If a female fruit fly is reared under optimal conditions, it can lay up to twice as many eggs as its total number of ovarioles each day (David and Merle, 1968). Therefore, if a female contains 20 ovarioles, it could lay 40 eggs in a 24-hour period under ideal conditions.

Types of Cell Death: Necrosis, Apoptosis, and Autophagy

Cell death is a natural biological process that occurs as a normal sequence of development and homeostasis. There are numerous types of cell death which are defined by the biochemical or morphological behavior of the cell. Severely injured cells may undergo necrosis, or unnatural cell death. Necrosis includes cell swelling, disruption of the integrity of the plasma membrane or organelle membranes, and chromatin digestion (Lockshin and Zakeri, 2001). In the final phase of necrosis, the cell will simply rupture and the intracellular contents will be released. Because necrosis is termed a disorderly type of cell death, there is no evidence that the proper cell signals are sent to phagocytes to engulf the dying cell. Therefore, it is much more difficult for the immune system to find and recycle dead cells that have undergone necrosis compared to those which have undergone apoptosis (Lockshin and Zakeri, 2001).

Physiological cell death has been divided into two main categories: apoptosis and autophagy. Unlike necrosis, both apoptosis and autophagy are programmed cell deaths that are under physiological control.

Apoptosis is a type of programmed cell death that was first described by morphological criteria and later by a distinctive and restricted form of degradation of DNA. Unlike necrosis, apoptosis possesses cellular mechanisms to properly dispose of cellular debris through phagocytes. The hallmarks of apoptosis include cell shrinkage, condensation and blebbing of the cytoplasm, changes to the cell membrane, nuclear fragmentation, chromosomal DNA fragmentation, and chromatin condensation (Bortner and Cidlowski, 2002). Cell shrinkage during apoptosis is the result of the loss of K^+ from the cell (Razik and Cidlowski, 2002) while the collapse of chromatin likely stems from

the degradation of DNA (Arends et al., 1990). Many of these characteristics are controlled by the activation of specialized restricted-target proteases called caspases (Salvesen, 2002).

The caspases are highly conserved from *Caenorhabditis* to *Drosophila* to mammals and were only discovered in 1993 (Yuan et al., 1993). The two general categories of caspases are initiator caspases (caspases 8 and 9) and effector caspases (caspases 3 and 7). Caspases 8 and 9 can be activated by an extracellular signal or by dramatic changes in the mitochondria of a cell, such as the release of cytochrome c into the cytoplasm (Suzuki et al., 2001). When the initiator caspases are activated, they in turn are able to activate the effector caspases leading to apoptotic activity. After proper signaling and activation, the effector caspases are able to attack crucial cytoplasmic proteins, strategic enzymes, and structural proteins (Suzuki et al., 2001).

Autophagy is a form of programmed cell death that involves the deterioration of the components of the cell through the use of lysosomes. Autophagy is usually employed by a cell to primarily remove large amounts of cytoplasm (Schmid and Muenz, 2007). The process of autophagy is generally not caspase-driven, and DNA destruction is not a priority (Klionsky and Emr, 2000). While there are several current studies regarding the processes of autophagy, there are many unanswered questions surrounding its regulatory mechanisms. Specific studies have demonstrated that autophagy plays a significant role in how a starving cell is able to breakdown cellular components in order to reallocate nutrients from unessential processes to more vital processes (Yorimitsu and Klionsky, 2005).

Autophagy and Apoptosis in Drosophila melanogaster

Current studies in *D. melanogaster* have made important advancements in understanding the processes of autophagy and apoptosis. Autophagy has been shown to not only be involved in physiological cell death in *Drosophila* but also to play a key role in mechanisms controlling cell survival (Berry and Baehrecke, 2007). Furthermore, it has been demonstrated that components of autophagy and apoptosis are shared between the two mechanisms (Berry and Baehrecke, 2007).

While apoptosis has been classified as type I programmed cell death (PCD), autophagy has been designated as a type II PCD (Schweichel and Merker, 1973). In addition, autophagy has been further defined by the presence of autophagosomes and autolysosomes within cells that are dying (Berry and Baehrecke, 2007). By using the salivary glands of *Drosophila*, Berry and Baehrecke demonstrated that PCD of the glands is triggered by the steroid hormone ecdysone. They found that the death of the salivary glands showed morphology of autophagy but were also aware that ecdysone played a role in caspase cascade activation in salivary glands which is associated with apoptosis.

To study autophagy, Berry and Baehrecke manipulated positive regulators of the class I phosphoinositide 3-kinase (PI3K) pathway, such as p110 and Akt, enabling them to investigate the inhibition of degradation of the salivary gland (Berry and Baehrecke, 2007). The investigators also demonstrated that autophagy gene (*atg*) mutants inhibit proper gland degradation. One major finding was that *atg* mutants still had active caspases which is a hallmark of apoptosis. They concluded that growth arrest and caspases contribute to autophagic cell death and that there may be other caspase-independent factors that are required for PCD of the salivary glands. Thus, components

of autophagy and caspases contribute to autophagic cell death of salivary glands (Berry and Baehrecke, 2007). This model of programmed cell death may apply to the larval fat body in the immature adult.

Cell Death Genes in Drosophila melanogaster

The genetic control of apoptosis has been extensively studied in *D. melanogaster*. The *Drosophila* apoptosis genes include *rpr (reaper), hid (head involution defect),* and *grim* (White et al., 1994). The structural similarity between Rpr, Hid, and Grim sits in a short N-terminal sequence known as the RHG motif (Wing et al., 2001). These proteins are capable of inducing cell death by inactivating the *Drosophila* inhibitor of apoptosis protein (Diap1). Rpr, Hid, and Grim interact with Diap1 in a RHG-dependent manner to promote Diap1 ubiquitination and degradation (Goyal et al., 2000).

Inhibitors of apoptosis (IAP) proteins are able to moderate apoptosis by directly binding to and inhibiting caspases (Goyal, 2001). There are two *Drosophila* IAPs known as Diap1 and Diap2. They contain two amino-terminal baculovirus inhibitor of apoptosis repeats (BIRs) that regulate binding to caspases (Hay et al., 1995). In addition, the Diap proteins have a carboxy-terminal RING finger domain that has E3 ubiquitin-ligase activity (Yang et al., 2000). Diap1 and Diap2 were two of the earliest cellular IAPs to be isolated as potent inhibitors of death induced by *rpr, hid* and *grim* (Hay, 2000). Studies have shown that Diap1 functions by inhibiting the processing and activation of caspases Dcp-1, Drice, and Dronc (Goyal et al., 2000; Muro et al., 2002). The precise function of Diap2 in cell death inhibition has not been well characterized (Quinn et al., 2000).

Stable Isotope Analysis: C3 versus C4 Plants

Numerous studies in *Drosophila* have focused attention into investigating the source and allocation of nutrients within the animal. It is clear that the larval feeding period is paramount for the success of the adult organism (Tu and Tatar, 2003; Goldsmith et al., 2004; Raguso et al., 2007), yet the mechanisms surrounding these events are currently not clearly understood. In the adult, larval energy stores must be allocated to both somatic and gonadal tissue in order to increase the animal's overall fitness. After larval energy stores are transferred and utilized by adult structures, the maintenance and maturation of these structures must then come from adult-derived nutrients. Stable isotopes have been used to gain new insight into the distribution and allocation of larval energy stores to adult gonadal and somatic tissues (Min et al., 2006).

Stable isotope analysis involves the identification of a ratio of stable elements in an investigated material and allows for direct inferences concerning diet, trophic level, and subsistence. Recent studies using stable isotope analysis have focused on the light elements of hydrogen, carbon, nitrogen, oxygen and sulfur in human and animal bone, tooth enamel, and hair to determine the diets and water sources of an organism (Webb et al., 1998).

One important use for isotope analysis is to identify the amounts of carbon isotopes within a tissue in order to determine the principal diet of an animal (Van der Merwe, 1982). On earth, 99 percent of all carbon exists in the Carbon 12 (^{12}C) form which has 6 protons and 6 neutrons. Most of the other one percent exists in the slightly heavier Carbon 13 (^{13}C) form containing 6 protons and 7 neutrons (Starr, 2006). The ratio of these isotopes in plants differs according to their photosynthetic mechanism. In C3

carbon fixation, the 3-carbon organic compound phosphoglyceric acid (PGA) is the first stable intermediate of the Calvin-Benson cycle. In C4 carbon fixation, the 4-carbon oxaloacetate forms as a stable intermediate in reactions that fix carbon twice before the Calvin-Benson cycle (Starr, 2006). Examples of C3 plants include sugar beets, rice, and wheat, while C4 plants include sugarcane, corn, and millet. In C4 carbon fixation, the heavier ^{13}C isotope is less depleted than in C3 carbon fixation. Therefore, C4 plants naturally have a higher fraction of ^{13}C than C3 plants (Fischer et al., 2004). This naturally occurring difference between these two types of plants may serve as a tool to monitor larval- versus adult-derived nutrients in holometabolous insects. In other words, if one can determine the ratio of ^{12}C to ^{13}C in an animal or animal tissue, then one can figure out what type of plant was incorporated into its diet.

CHAPTER 2

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, molasses and corn syrup 36.3 ml/l each, yeast 18 g/l, soy flour 11 g/l, ethanol 12 ml/l, agar 6 g/l, propionic acid 5.2 ml/l, and niapagen 1.2 g/l) 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, Indiana, 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, Connecticut., 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 (Cooley, unpublished).

I used the GAL4/UAS system of Brand and Perrimon (1993) to restrict expression of GFP to larval fat-body cells. 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).

The *Lsp2-GAL4* transgene contains the *Lsp2* promoter and recapitulates the expression pattern of the endogenous *Lsp2* gene. *Lsp2* is expressed solely in larval fat-body cells beginning early in the third larval instar (B. Hassad, personal communication to FlyBase). The Gal4 protein encoded by *Lsp2-GAL4* is produced only in the larval fat-body cells in the 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 to *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. In all experiments, the two parental stocks in which normal programmed cell death occurs were used as controls.

Quantitative analysis of larval fat cells and ovaries

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 into1x 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 x 75mm glass slide. Cells were examined by light and fluorescent microcopy 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 x 75mm glass slides using GelMount (Sigma, St Louis, Missouri, USA). GFP fluorescence was measured using a Typhoon 8600 Variable Mode Imager and the intensity of the phosphoimage (in pixels) quantified using ImageQuant software.

Ovaries were dissected from females in Dulbecco's phosphate buffered saline (DPBS) on a 25x75 mm glass slide. Dissected ovaries were examined by light microscopy, and photographs were taken using a Canon A620 digital camera coupled to a Zeiss Stemi 2000-C microscope and Canon Zoom Browser EX photo 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 ten and starved in 47mm 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 in chapter 3 show 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 carried out 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 1x DPBS. Cells were analyzed within an hour after slide preparation.

Stable isotope studies

Animals for stable isotope analysis were reared on either a cane sugar- or beet sugar-based diet as larvae (corn meal, 42.6 g/l; beet or cane sugar, 68.2 g/l; yeast, 23.9 g/l; agar, 7.9 g/l; and propionic acid, 4.5 ml/l), supplemented with dry yeast.. Newly eclosed (0-2 hour old) female adults were collected and were maintained on the same diet or switched to the other sugar-based diet. Thus four different feeding regimes were used in this study (larval diet:adult diet): cane:cane; cane:beet; beet:beet; and beet:cane. Ovaries from 1 to 7 day old virgin female adults were dissected on a 25x75 mm glass slide in a drop of DPBS. Approximately 0.5 mg of ovaries (6 to 36 per sample) were placed in 5x9 mm pressed-tin capsules (Costech Analytical Technologies; Valencia, California, USA; No. 041061) and dried at 50°C for 48 hours. Dry weight for each sample was then determined using a Cahn C-30 microbalance to a precision of 1μg. Samples were analyzed using a Costech NA 2000 Elemental Analyzer coupled with Delta V Plus mass spectrometer by the Las Vegas Isotope Science Laboratory (LVIS; University of Nevada, Las Vegas). Isotope ratios ($\delta^{13}C$) are reported in parts per million values relative to Peedee Belemnite (Werner and Brand, 2001).

Initiation of egg laying and egg laying capacity

To establish when egg laying is initiated and to determine total egg laying capacity, recently eclosed females (0-2 hours) were collected and placed into individual wells of an 8 x 11.6cm 24-well food plate Fly Condo ™ (Genesee Scientific; San Diego, California, USA; No. 59-110) containing grape agar (Genesee Scientific; San Diego, California, USA) and supplemented with yeast paste. Groups of 12 females per trial were observed every 12 hours for egg deposition. The number of eggs deposited in each 12

hour period was recorded for 7 to 10 days. Similar experiments were also carried out with newly eclosed females paired with 2 males.

Protein, triglyceride and carbohydrate assays

Energetic substrates (carbohydrates, proteins, and lipids) were assayed in triplicate using standard protocols. Adult females were homogenized in a lysis solution containing detergent to solubilize lipids (1% NP-40, 0.5% deoxycholic acid, 0.1% Triton-X 100, 100 mM NaCl, 0.1 mM $CaCl_2$, 2 mM $MgCl_2$, pH 7.6). Triacylglyceride levels were measured using a commercial serum triglyceride kit (Sigma; St. Louis, Missouri USA; cat no. TR0100-1KT), and protein was quantified using the bicinchinonic acid method (Smith, 1985). Carbohydrates (glycogen and trehalose) were digested with amyloglucosidase and quantified with a blood glucose kit (Pointe Scientific; Canton, Michigan, USA; kit no. G7521).

CHAPTER 3

THE ROLE OF LARVAL FAT CELLS IN ADULT *DROSOPHILA MELANOGASTER*

Abstract

In the life history of holometabolus 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 which 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 hours to 72 hours. 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.

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 hours in search of a pupation site (Riddiford, 1993). After eclosion, the newly-emerged adult remains inactive for approximately 8 hours until the wings expand and the cuticle tans (Chiang, 1963; Aguila and Hoshizaki, unpublished). 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, while 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, on the other hand, 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 in 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 hours, 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, molasses and corn syrup 36.3 ml/l each, yeast 18 g/l, soy flour 11 g/l, ethanol 12 ml/l, agar 6 g/l, propionic acid 5.2 ml/l, and niapagen 1.2 g/l) 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, Indiana, 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, 2004; Morin et al., 2001) and was generously provided by L. Cooley (Yale University, New Haven, Connecticut., 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, 2004) and is within *chickadee* but on the opposite strand, *i.e.*, in the opposing reading frame (Cooley, unpublished).

In separate experiments, we used the GAL4/UAS system of Brand and Perrimon (1993) to restrict expression of GFP to larval fat-body cells. 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 down stream 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* on to 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 to *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 into1x DPBS (52mM NaCl; 40mM KCl; 10mM HEPES; 1.2mM $MgSO_4$; 1.2mM $MgCl_2$; 2mM Na_2HPO_4; 0.4mM KH_2PO_4; 1mM $CaCl_2$; 45mM sucrose; 5mM glucose, pH 7.2) on a 25 x 75mm glass slide. Cells were examined by light and fluorescent microcopy 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 x 75mm glass slides using GelMount (Sigma, St Louis, Missouri, 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 ten and starved in 47mm 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 carried out 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 1x DPBS. Cells were analyzed within an hour after slide preparation.

Results

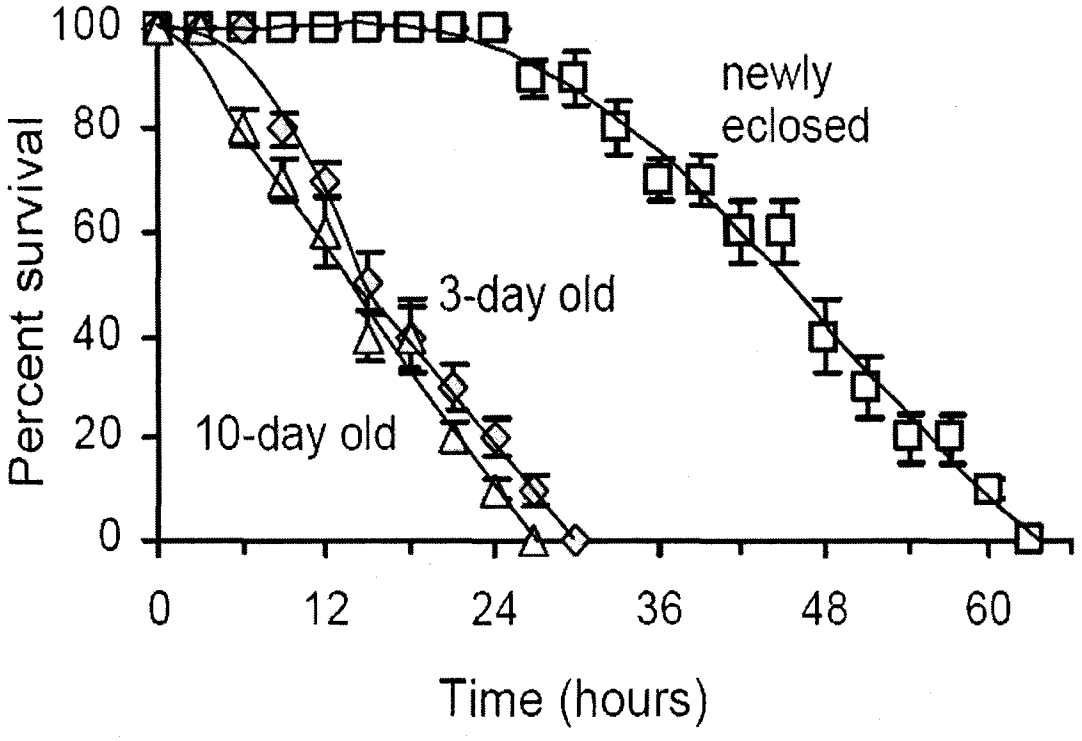
Adults flies starved upon eclosion are more resistant to starvation than older flies

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 (0-10min) and either immediately tested for starvation resistance, or aged on food supplemented with yeast before testing. We found that newly eclosed females adults were more resistant to starvation (LD_{50} = 45hr) than 3 or 10 day-old animals (LD_{50} = 16hr and 14hr, respectively; Fig 1). These data support the idea that the free-floating fat cells represent a significant energy source.

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 defining the underlying basis of the higher starvation resistance of newly eclosed adults.

Figure 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.



To experimentally establish the origin of these cells in the young adult, we took advantage of a Green Fluorescent Protein (GFP) protein trap line for a polytene chromosome-associated protein (Andres, 2004). Polytene chromosomes are a hallmark of larval tissues including the fat body. We used this cell marker to distinguish between adult tissues which contain mitotic chromosomes from 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 to the physical number of larval fat cells obtained by dissection of individual animals (Fig. 4); GFP fluorescence was proportional to the number of the 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 hours post eclosion, 50% of the larval fat cells have undergone cytolysis (Fig. 4 and see Fig 6.).

Figure 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.

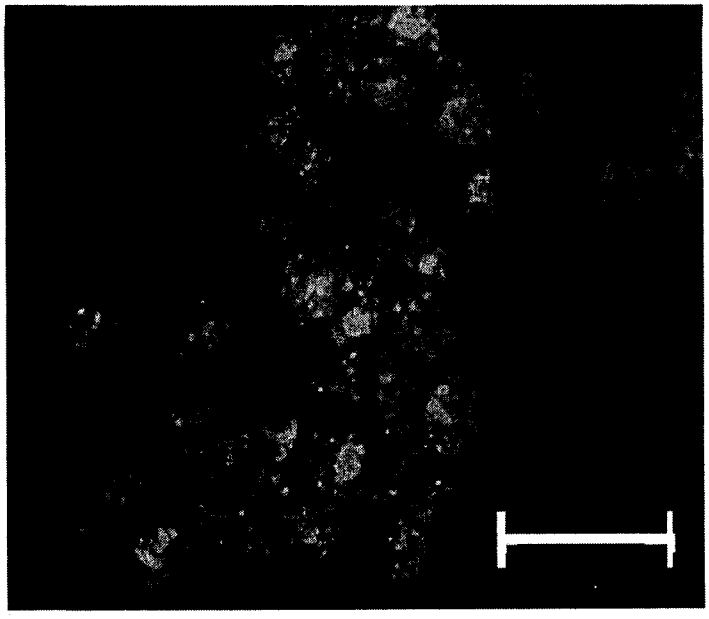
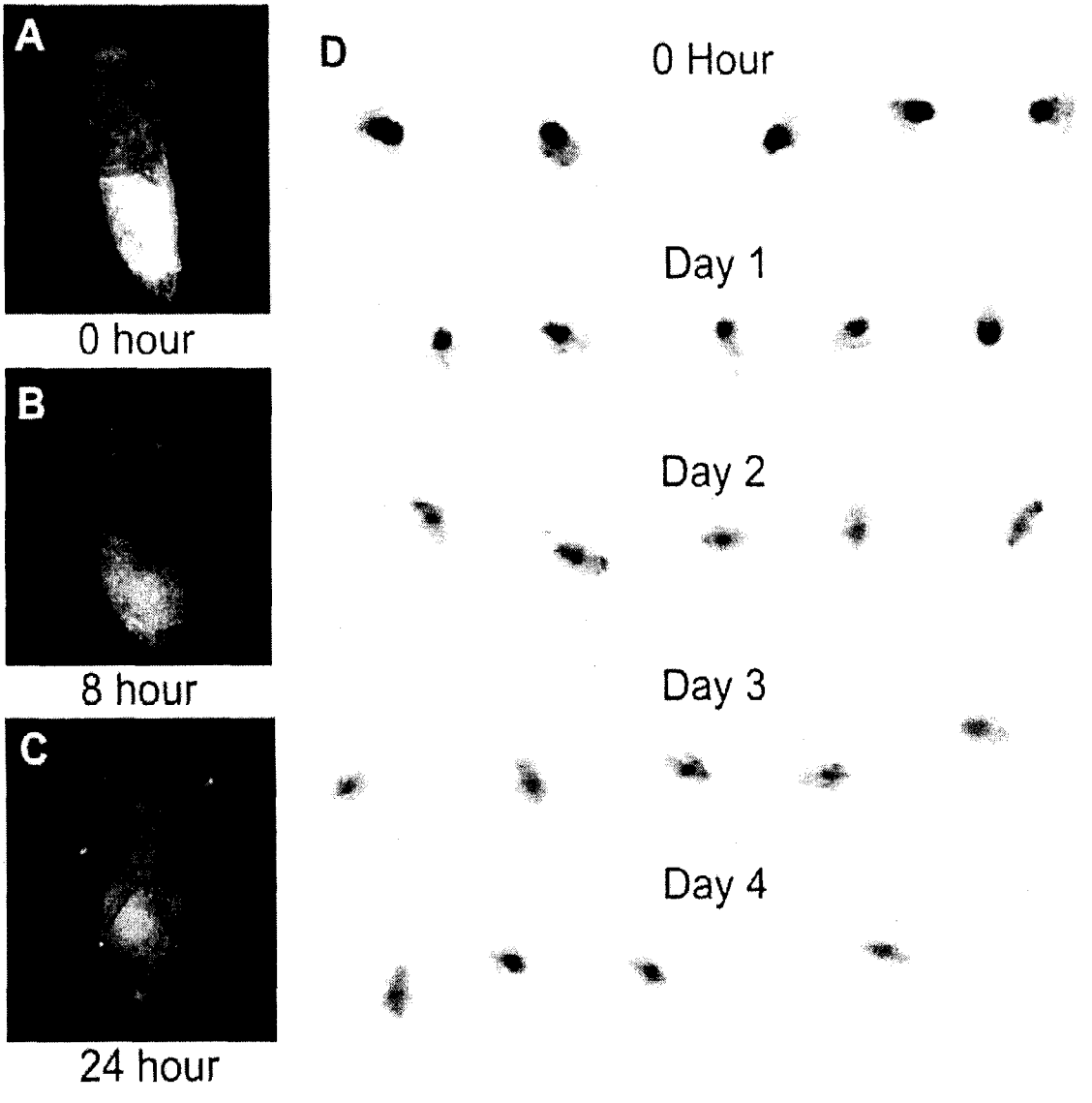


Figure 3. Whole-mount adults used for GFP-based measurement of larval fat cells. (A–C) Fluorescent images of whole-mount *Lsp2-Gal4::UAS-GFP* aged adult females. GFP labeled larval fat cells are prominent in the abdomen. (D) Phosphoimage of whole-mount *Lsp2-Gal4::UAS-GFP* aged adult females used to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



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 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} = 58hr) than 3 or 10 day old adults, (LD_{50} = 26hr and 20hr, 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, starvation did not affect the rate of larval fat-cell cytolysis; within 8.5 hours 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

Figure 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.

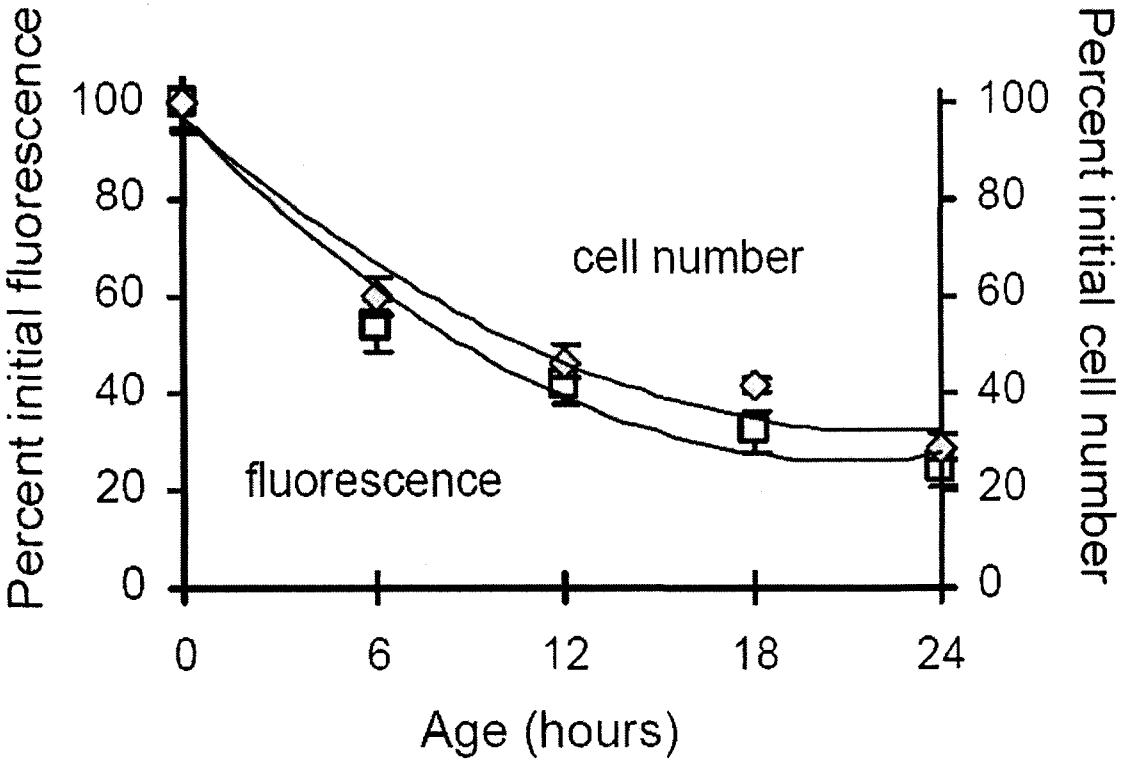


Figure 5. Starvation resistance of *Lsp2-Gal4::UAS-GFP* adults decreases with age. Starvation resistance was measured by percentage survival of adult females in groups of 10 flies. Newly eclosed *Lsp2-Gal4::UAS-GFP* adults (*N*=8 groups of 10) (squares), 3 day-old *Lsp2-Gal4::UAS-GFP* adults (*N*=14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

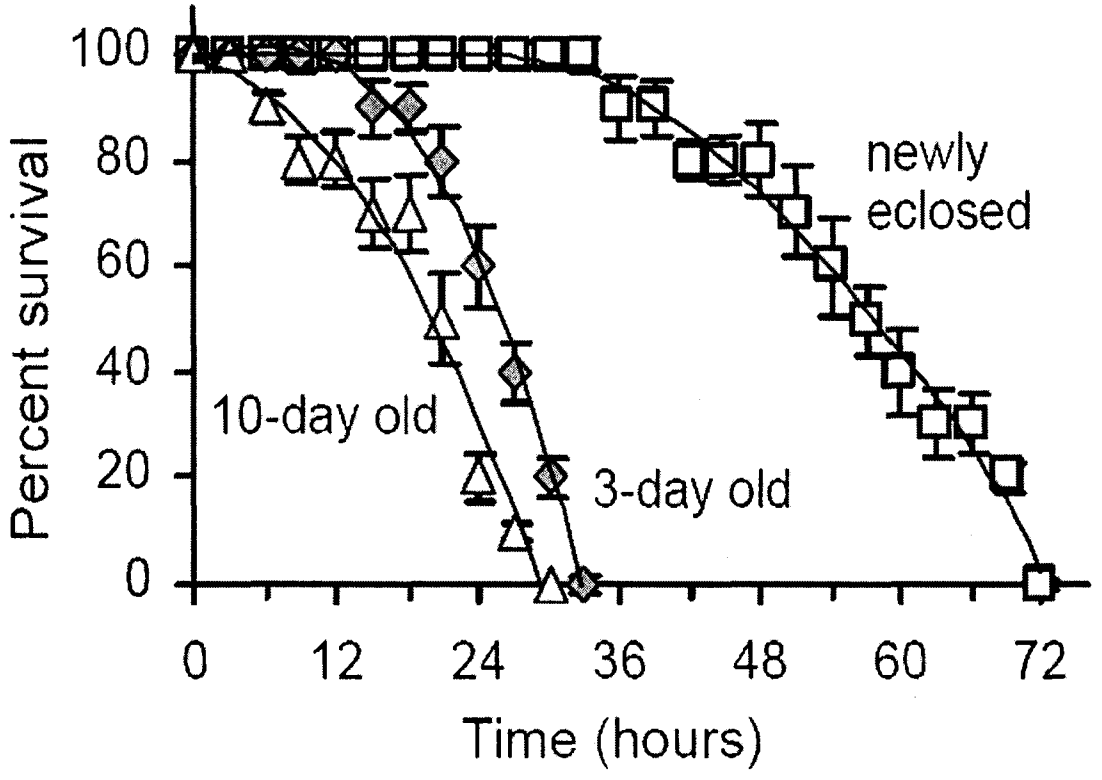
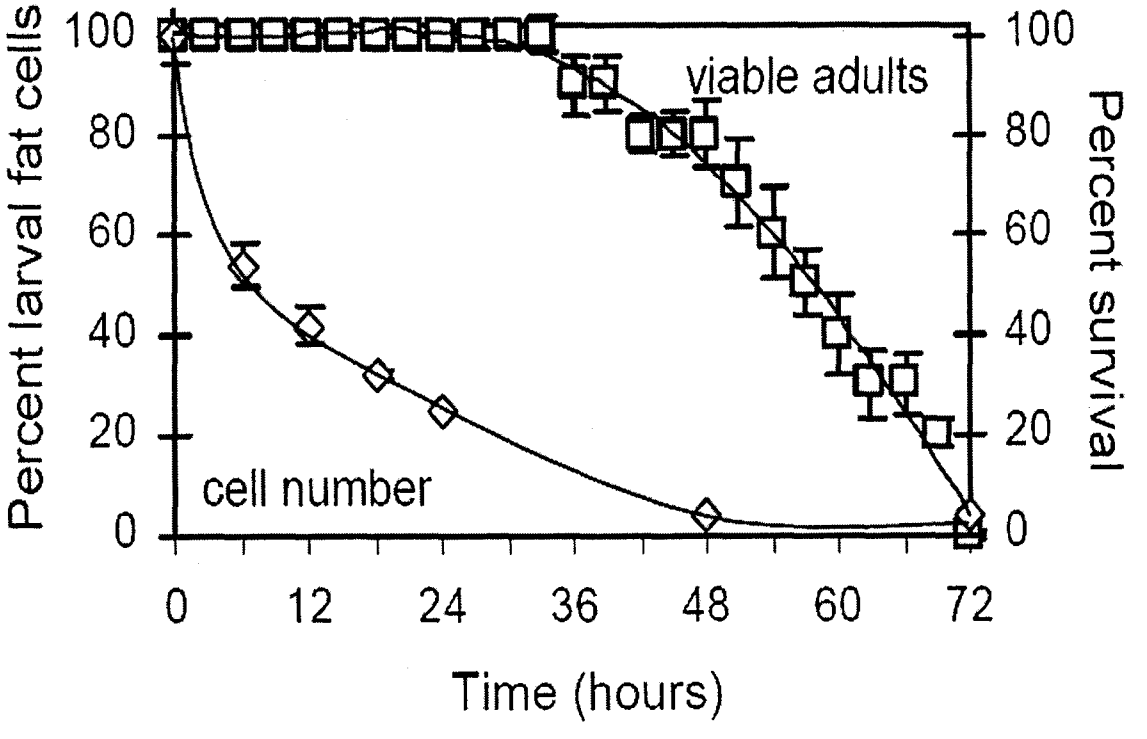


Figure 6. Larval fat-cell number and starvation resistance in newly eclosed adults. Larval fat-cell number measured for *Lsp2-Gal4::UASGFP* adult females using the GFP-based assay (N=25–36 per time point), and compared with the percentage survival of newly eclosed *Lsp2-Gal4::UAS-GFP* starved adult females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



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 equal number of fat cells were 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} = 57hr to LD_{50} = 82hr (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 hours post eclosion, when ~70% of the fat cells have normally undergone cytolysis, only 38% of 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 hours, 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 hours experimental adults began to succumb to starvation while ~22% of the larval fat cells remained (compare Fig. 7A with Fig.8).

Figure 7. Larval fat cells persist in aged adults when cell death is blocked. (A) *In situ* fat cell number from *Lsp2-Gal4::UAS-GFP/UASdiap1* adult females in which cell death is blocked (*N*=15–20 individuals per time point, filled bar) compared with *Lsp2-Gal4::UASGFP* 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) GFPfluorescence 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.

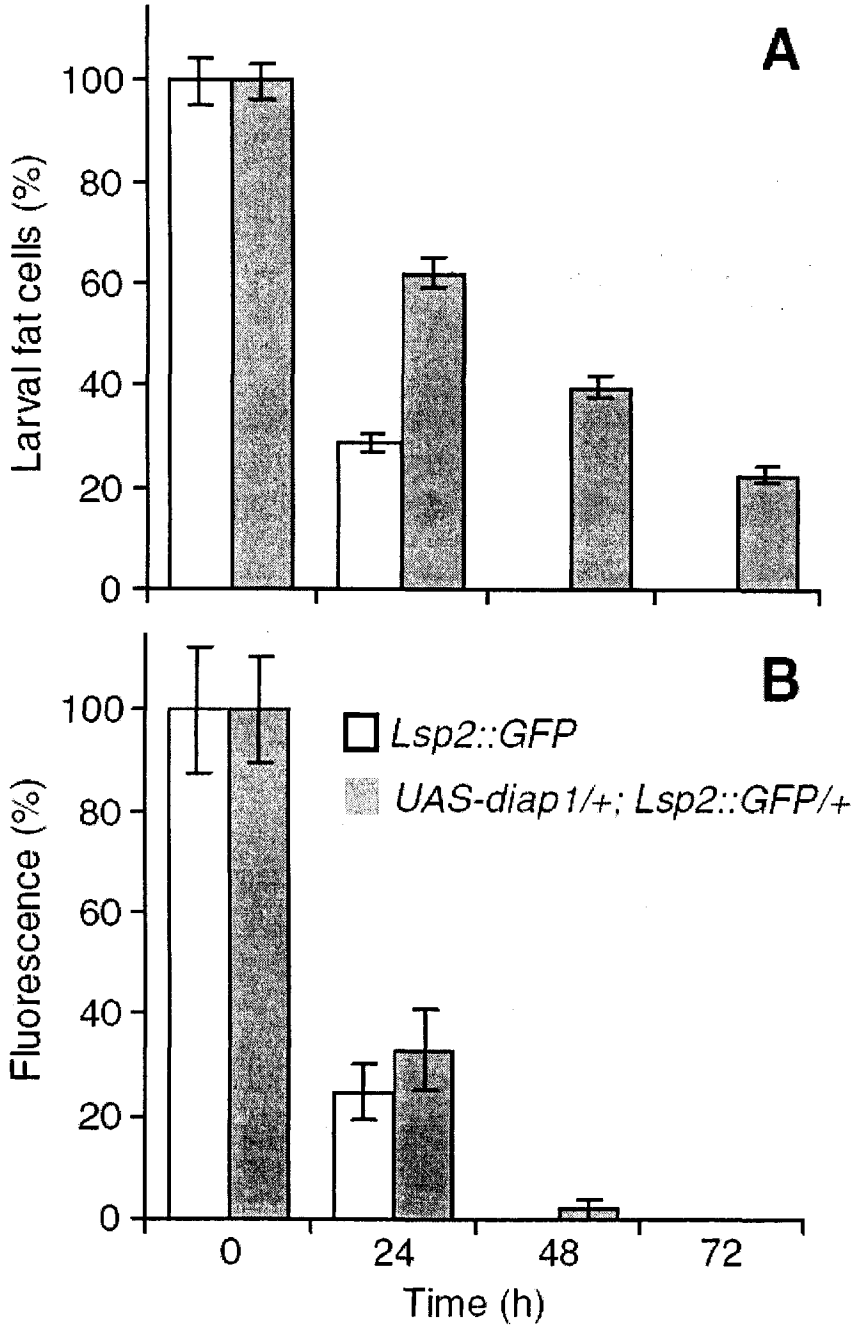
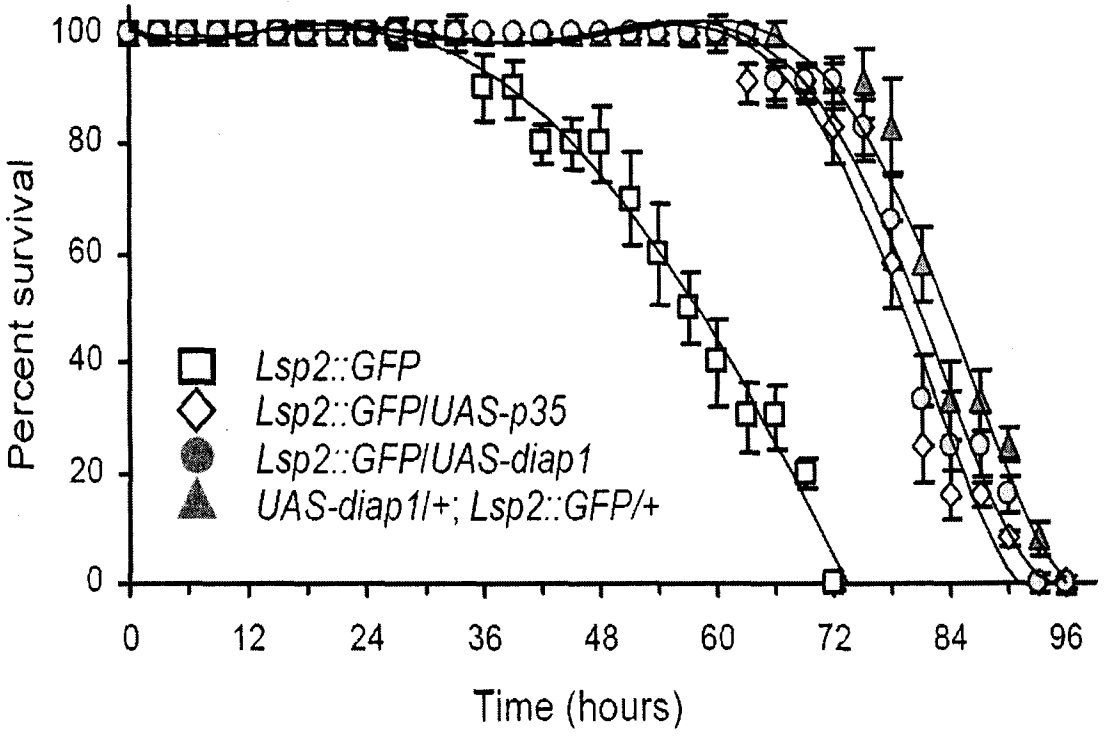

A
Larval fat cells (%)
100
80
60
40
20
0
B
Fluorescence (%)
Lsp2::GFP
UAS-diap1/+; Lsp2::GFP/+
0
24
48
72
Time (h)

Figure 8. Starvation resistance increases in adults when larval 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::UASGFP/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.



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 must relocate to new feeding sites.

Larval fat cells in the adult

Through the use of cell markers, we have demonstrated using cell markers 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; SD = 49), which is in contrast to the 1052 cells (n = 8; SD = 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. In 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 report). 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., 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 approximately 1250 cells. Our average 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 hours 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 in 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 where 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 hours. 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 hours 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, on the other hand, is a membrane trafficking process involving autophagosomes which 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 hall marks 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) while 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, while 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 which leads to successful reproduction. The larval fat cells therefore may 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 times scales 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 accumulate 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), though not all (Baldal et al., 2004). A likely explanation for this phenomenon is allocation of resources to reproduction during early adulthood. 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 to 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 over 24 hours. This increase is not due to 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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CHAPTER 4

LARVAL FAT CELLS DRIVE FEMALE REPRODUCTION IN *DROSOPHILA MELANOGASTER*

Abstract

Holometabolous insects possess a phenomenal life cycle that is divided into unique larval and adult stages closely linked to feeding and non-feeding periods. During the larval stages, the attention of the animal is directed primarily to feeding, in order to accumulate energy stores in the larval fat body for later use in the pupal and adult stages. In previous studies, we reported that energy stores acquired during larval development are later transferred to the adult *via* the larval fat cells (Aguila et al., 2007) and suggested that nutrients accumulated by the larval fat cells followed by programmed cell death in the adult is important for female reproduction. We demonstrated that newly eclosed females are three times more starvation resistant than aged females due to the presence of the larval fat cells. Furthermore, the inhibition of programmed cell death in the larval fat cells resulted in a distinct advantage in starved females, where disruption of programmed cell death resulted in four times more starvation resistance than aged adult females (Aguila et al., 2007). While it is apparent that larval fat cells serve as an important energy reservoir in the adult female, these data do not explain why females in which fat-cell energy stores are released by programmed cell death are more sensitive to starvation than females in which death of the larval fat cells is delayed.

We report here that the "quick" release of energy stores from larval fat cells by cell death promotes the rapid maturation of the ovaries and has an important role in establishing female fecundity. By using stable carbon isotopes, we followed the acquisition of larval nutrients by the adult ovaries and demonstrate that this acquisition is driven by the programmed cell death of the larval fat cells. Furthermore, we demonstrate that in the absence of programmed cell death, ovary development is delayed although normal ovary size is achieved by day 4 of adult life. Concomitant with the delay in ovarian development, initiation of egg laying is delayed by 24 hours, and the egg laying capacity is depressed by 63% and 36% in virgin and mated females, respectively. This depression is not the result of differences in the initial energy accumulation from larval feeding or differences in the size of the adult. We discuss the implications of these results in terms of the importance of the larval reserves in establishing female fecundity, the trade-off between growth and size, and life history traits.

Introduction

In the life history of holometabolous insects, distinct developmental stages are tightly linked to feeding and non-feeding periods. During the last three days of larval development in *D. melanogaster*, the animal will attain a 200-fold increase in mass (Church and Robertson, 1966) where the nutrient reserves are primarily accumulated in the larval fat body. Before metamorphosis, the larva stops feeding and a 12-24 hour "wandering" phase begins in which the animal searches for a suitable pupation site (Riddiford, 1993). After eclosion, the newly-emerged adult remains inactive for approximately eight hours until the wings expand and the cuticle tans (Chiang, 1963;

Edgecomb et al., 1994; J.R.A and D.K.H., unpublished data). Larvae must therefore acquire enough nutrients not only to fuel the developmental re-organization of the pupa but also to survive the final larval and early adult periods.

In *Drosophila*, metamorphosis is characterized by an extraordinary transformation from the larval to the adult state. The imaginal cells proliferate to give rise to the adult tissues, while the larval tissues are degraded through the process of autophagic programmed cell death (Lee and Baehrecke, 2001). A striking exception to this loss of larval tissues is the fat body, which undergoes tissue dissociation into individual cells (Nelliot et al., 2006). These larval fat cells survive as independent cells throughout metamorphosis and function as a nutritional reservoir to fuel the re-architecture of the pupa to the adult state. The larval fat cells are carried forward into the young adult and provide energy for the development of adult somatic and gonadal tissues (Aguila et al., 2007).

In previous work (Aguila et al., 2007), we demonstrated that energy stores acquired during larval development are later transmitted to the adult *via* the larval fat cells. The larval fat cells normally undergo programmed cell death in the immature adults and within 24 hours 85% of the fat cells are lost, releasing their contents into the hemolymph. Newly-eclosed adult females with their full complement of larval fat cells are three times more starvation resistant than mature females in which the larval fat cells have undergone cell death. Furthermore, genetic inhibition of programmed cell death of the larval fat cells further increases starvation resistance by four times (Aguila et al., 2007). It is clear that the larval fat cells serve as an important energy reservoir in the adult female, yet these data do not explain why energy stores retained within the fat cells allow

for increased starvation resistance when compared to females in which the fat-cell energy stores have been released by programmed cell death.

In this study, we address this conundrum and test the hypothesis that the "quick" release of energy stores from the larval fat cells *via* programmed cell death drives the rapid maturation of the ovaries. We conducted stable isotope studies to measure the relative contributions of larval- versus adult-derived nutrients into the ovaries. To distinguish between larval- and adult-derived nutrients, we took advantage of the naturally occurring differences in the ratio of ^{13}C to ^{12}C in sugars derived from sugar beets versus sugar cane. The sugar beet undergoes C3 photosynthesis and therefore contains a lower ratio of ^{13}C to ^{12}C than sugar derived from sugar cane, which utilizes C4 photosynthesis (Starr, 2006). Stable isotopes provide a means to estimate the relative contributions of different dietary sources to the tissues. (O'Brien et al., 2002; Fischer et al., 2004; Min et al., 2006). By feeding larvae and adults diets containing beet or cane sugar which differed in stable carbon isotope ratios, we were able to track larval- versus adult-derived nutrients into the ovaries of adult animals. We tracked nutrients in wild-type animals and animals in which larval-fat cell death was genetically blocked in order to test the role of caspase-induced programmed cell death in mobilizing larval nutrients into the adult ovaries.

We found that larval nutrients make up ~42% of the carbon in two day-old ovaries and in the absence of programmed cell death of the larval fat cells, uptake of larval nutrients is reduced in the ovary and ovarian development is delayed. Furthermore, the initiation of egg laying and egg laying capacity was depressed. This depression was not the result of differences in the initial energy accumulation from larval feeding or

differences in the size of the adult. Our data support the idea that larval nutrition and energy accumulation are important contributors to ovarian development and fecundity.

Materials and Methods

Drosophila husbandry and genetic crosses

All flies were raised at 25°C on a corn meal-sugar-yeast medium (corn meal, 42.6 g/l; beet or cane sugar, 68.2 g/l; yeast, 23.9 g/l; agar, 7.9 g/l; and propionic acid, 4.5 ml/l), supplemented with dry yeast.

Genetically constructed flies were used in ovary mass, stable isotope, and fecundity assays. To block cell death in the larval fat cells, the cell death inhibitor gene, *Drosophila inhibitor of apoptosis 1* (*diap1*), was employed. Construction of flies with the genotype *y w; P{Lsp2-GAL4.H}, P{w+mc=UAS-n-syb.eGFP}3* (abbreviated *Lsp2-GAL4::UAS-GFP*) is described in Aguila et al., 2007. Ectopic expression of *diap1* was achieved using the GAL4/UAS system (Brand and Perrimon, 1993). Individuals carrying the diap UAS transgene, *P{w[+mC]={UAS-DIAP1.H}1 (abbreviated UAS-diap)*, Bloomington Stock Center; Bloomington, Indiana USA) were crossed to *Lsp2-GAL4::UAS-GFP* to drive ectopic expression of *diap1* to the larval fat cells, to inhibit cell death in these cells (Aguila et al., 2007). In all experiments, the two parental stocks in which normal programmed cell death occurs were used as controls.

Photography

Ovaries were dissected from females in 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 x 75 mm glass slide. Ovaries were examined by light microscopy, and photographs were taken using a Canon A620 digital camera coupled to a Zeiss Stemi 2000-C microscope and Canon Zoom Browser EX photo software.

Stable isotope studies

Animals for stable isotope analysis were reared on either a cane sugar- or beet sugar-based diet as larvae. Newly eclosed female (0-2 hour old) adults were collected and were maintained on the same diet or switched to the other sugar-based diet. Thus four different feeding regimes were used in this study (larval diet:adult diet): cane:cane; cane:beet; beet:beet; and beet:cane. Stable isotope analyses of ovaries from 1 to 7 day old virgin female adults were carried out. Ovaries were dissected on a 25 x 75 mm glass slide in a drop of DPBS. Approximately, 0.5 mg of ovaries (6 to 36 per sample) were placed in 5x9 mm pressed-tin capsules (Costech Analytical Technologies; Valencia, California, USA; No. 041061) and dried at 50°C for 48 hours. Dry weight for each sample was then determined using a Cahn C-30 microbalance to a precision of 1μg. Samples were analyzed using a Costech NA 2000 Elemental Analyzer coupled with Delta V Plus mass spectrometer by the Las Vegas Isotope Science Laboratory (LVIS; University of Nevada, Las Vegas). Isotope ratios ($\delta^{13}C$) are reported in parts per million values relative to Peedee Belemnite (PDB). Historically, the so-called PeeDee Belemnite was agreed to be used as a common reference to define the zero-point of the carbon isotope (Werner and Brand, 2001).

Initiation of egg laying and egg laying capacity

To establish when egg laying is initiated and to determine total egg laying capacity, recently eclosed females (0-2 hours) were collected and placed into individual wells of an 8 x 11.6cm 24-well food plate Fly Condo ^{TM} (Genesee Scientific; San Diego, California, USA; No. 59-110) containing grape agar (Genesee Scientific; San Diego, California, USA) and supplemented with yeast paste. Groups of 12 females per trial were observed every 12 hours for egg deposition. The number of eggs deposited in each 12 hour period was recorded for 7 to 10 days. Similar experiments were also carried out with newly eclosed females paired with 2 males.

Protein, triglyceride and carbohydrate assays

Energetic substrates (carbohydrates, proteins, and lipids) were assayed in triplicate using standard protocols. Adult females were homogenized in a lysis solution containing detergent to solubilize lipids (1% NP-40, 0.5% deoxycholic acid, 0.1% Triton-X 100, 100 mM NaCl, 0.1 mM $CaCl_2$, 2 mM $MgCl_2$, pH 7.6). Triacylglyceride levels were measured using a commercial serum triglyceride kit (Sigma; St. Louis, Missouri USA; cat no. TR0100-1KT), and protein was quantified using the bicinchinonic acid method (Smith, 1985). Carbohydrates (glycogen and trehalose) were digested with amyloglucosidase and quantified with a blood glucose kit (Pointe Scientific; Canton, Michigan, USA; kit no. G7521).

Results

We previously demonstrated that the larval fat cells persist into the adult stage and that these cells can serve as an important nutrient reservoir for the immature adult to survive starvation (Aguila et al., 2007). Newly-eclosed females with their normal

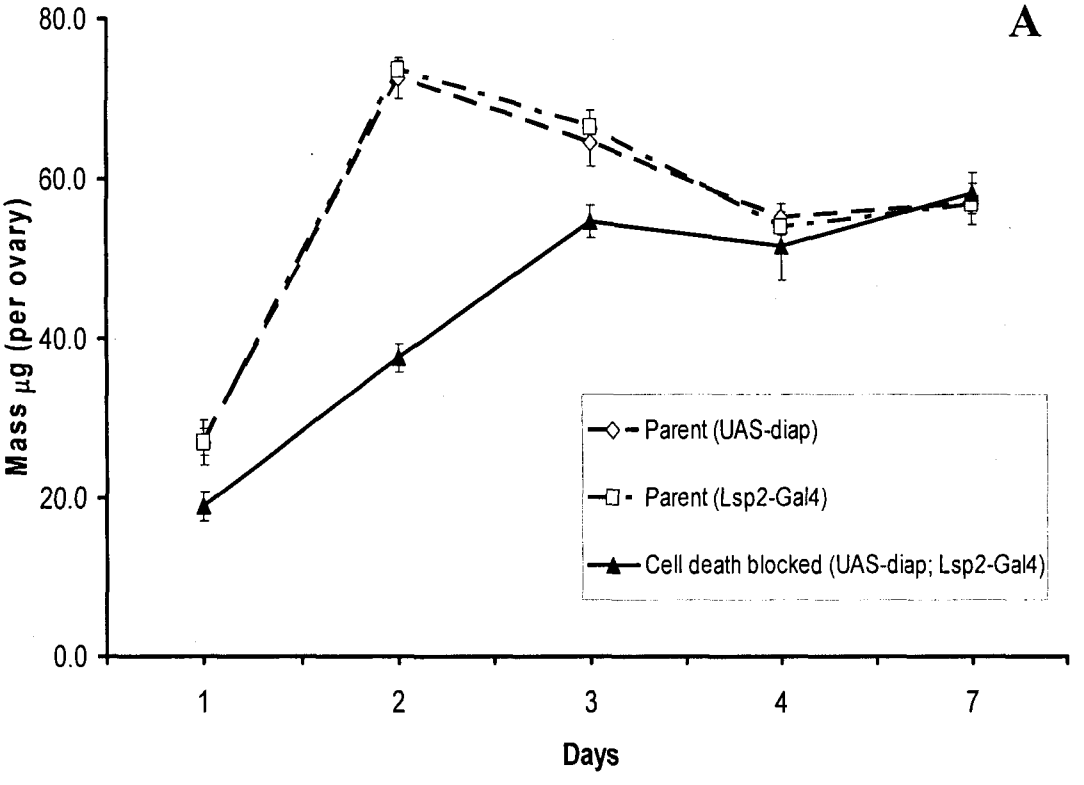
complement of larval fat cells are three times more starvation resistant than aged females which lack larval fat cells. Furthermore, starvation resistance is enhanced by four times in adult females in which normal caspase-induced programmed cell death is blocked in the larval fat cells. We hypothesized that in females, programmed cell death of the larval fat cells allows for the "quick" release of fat cell contents, i.e. lipids, carbohydrates, and protein that are used to drive the maturation of the ovaries.

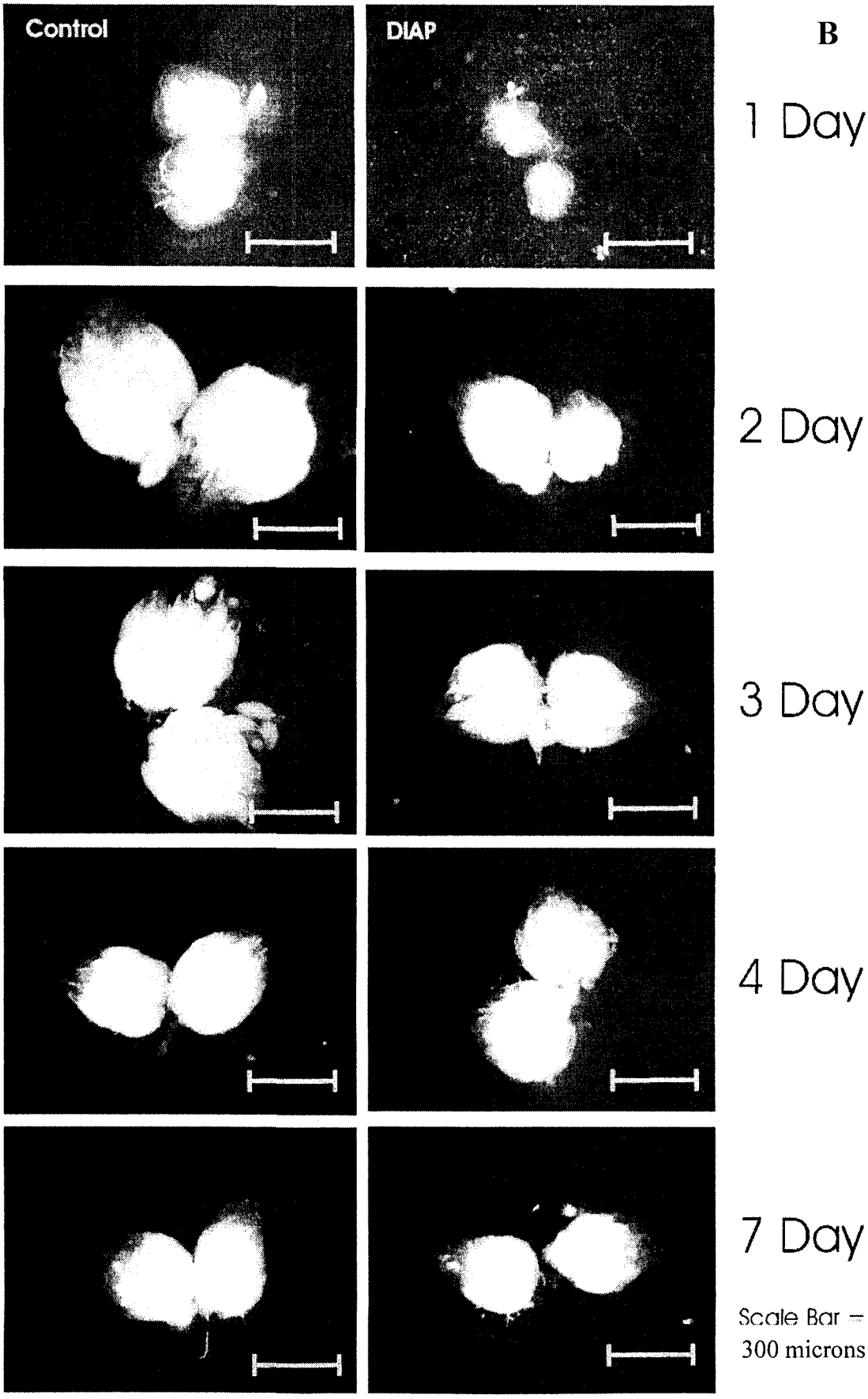
Ovarian Uptake of Larval Nutrients is Driven by Programmed Cell Death of the Larval Fat Cells

In order to test our hypothesis, we investigated if blocking the normal cell death of the larval fat cells would affect the mass or size of the adult ovary. To test this assertion, animals were grown on a normal cane:cane diet and the growth of the ovaries was monitored at 24 hour intervals. Qualitative differences in ovary mass were easily detected (Fig. 9B). In the parental line, *Lsp2-GAL4::UAS-GFP,* ovaries increased dramatically in size to 73 $\pm$ 3 μg/ovary by day 2 and then exhibited a decline in mass that stabilized at 55 $\pm$ 2 μg/ovary by day 4. In contrast, ovaries from the F1 females are already underdeveloped by day 1, never reached the peak mass (37 $\pm$ 2 μg/ovary) exhibited by day 2 in the ovaries of the parental line, but achieved the same mass (52 $\pm$ 4 μg/ovary) as the ovaries from the parents after day 4 (Fig. 9A).

To further test our hypothesis that the larval fat cells serve as carriers of energy for the development of the ovaries, we used stable isotopes to track ovary acquisition of nutrients acquired during larval and adult feeding. We predicted that the normal death of the larval fat cells allows for rapid uptake of larval nutrients by the ovaries in the

Figure 9. Ovaries from adult wild-type and fat cell-death inhibited females. Cell-death inhibited *(Lsp2-GAL4/UAS-diap)* animals' ovaries have less mass (A) and are visibly smaller (B) compared to wild-type animals from days 1-3.

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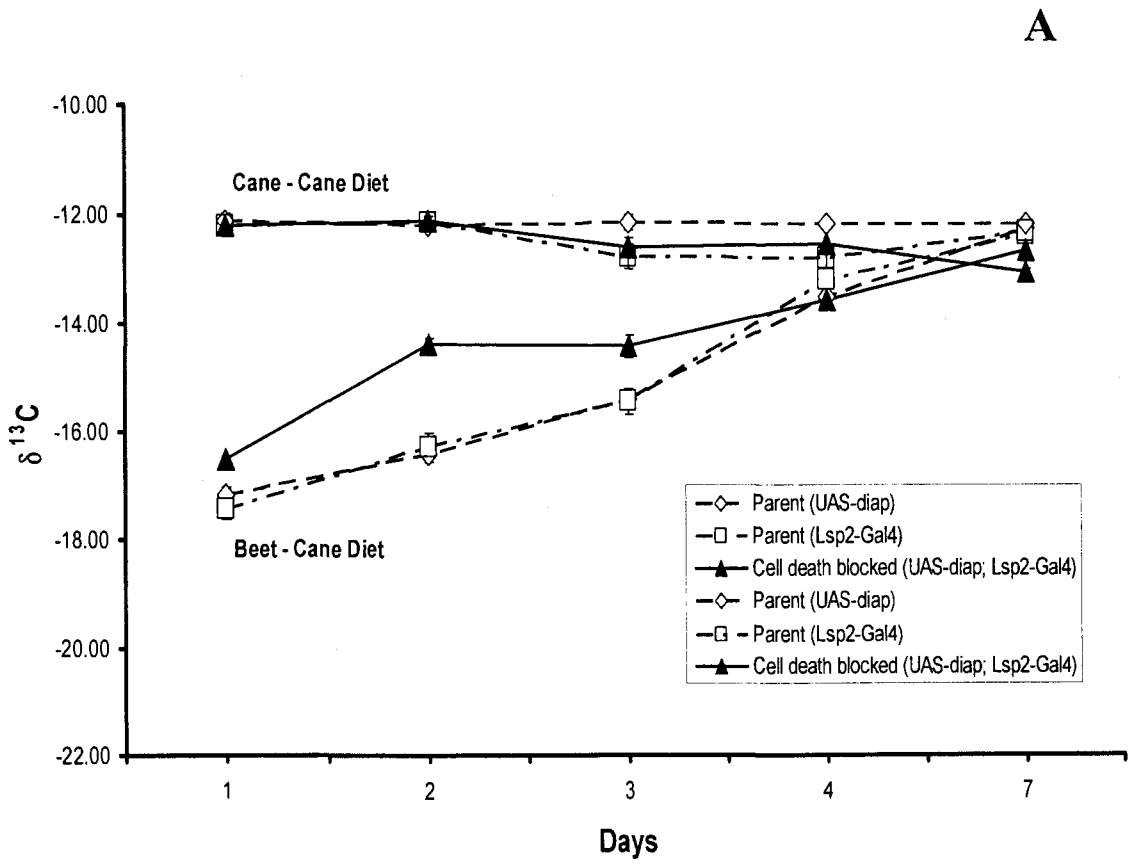
immature adult and that in the absence of cell death only minor uptake of larval-derived nutrients is possible. To inhibit cell death of the larval fat cells, we used the GAL4/UAS system to express the cell death inhibitor gene *diap1* specifically in the fat cells (see material and methods and Aguila, et al., 2007 for details).

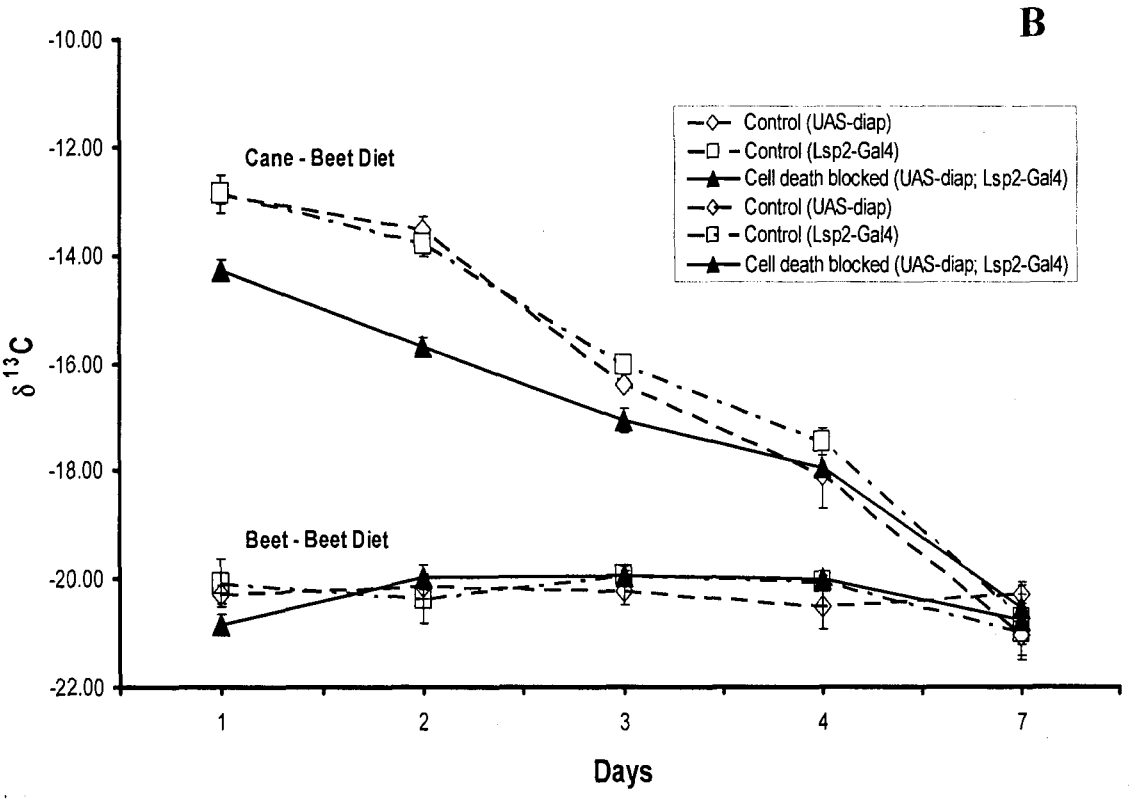
To track larval- and adult-derived nutrients, larvae were raised on a cane sugar-based diet, and newly-eclosed females were collected and either maintained on the larval diet (cane sugar) or switched to the beet sugar-based diet. These females are designated as cane:cane or cane:beet animals, respectively. Other larvae were grown on a beet sugar-based diet, and the newly eclosed females were either maintained on the larval diet (beet sugar) or switched to the cane sugar-based diet. These females are designated as beet:beet or beet:cane, respectively.

Ovaries were dissected from aged individuals from the parental lines, *Lsp2-GAL4::UAS-GFP* (a fat body specific Gal4 driver that carries a GFP cell marker) or *UAS-diap* (the UAS responder line), and from the F1 offspring (*UAS-diap/+; Lsp2-GAL4::UAS-GFP/+*) in which programmed cell death is blocked in the larval fat cells. The proportion of carbon derived from the larval and adult diet present in the ovaries was determined by mass spectrometric elemental analysis and expressed as $\delta^{13}C$, the ratio of ^{13}C to ^{12}C relative to Peedee Belemnite.

As expected, ovaries from the parental lines and the F1 offspring which were maintained on the cane:cane diet had a carbon isotope ratio of $\delta^{13}C = -12.4 \pm 0.29$, the carbon signature characteristic of cane sugar (Fig. 10A), while ovaries from the parental lines and the F1 offspring reared on beet:beet diet had a carbon isotope ratio of $\delta^{13}C$

Figure 10. Stable isotope analysis of adult ovaries. Stable isotope analysis was conducted on ovaries dissected from adult animals. Female adults were given one of four different diet regiments (larval diet:adult diet): cane:cane; cane:beet; beet:beet; and beet:cane. (A). Larvae reared on cane-sugar medium, then reared on cane as adults (top lines) or beet (bottom lines). (B). Larvae reared on a beet-sugar medium, then reared on beet as adults (bottom lines) or cane (top lines). Points represent average $\delta^{13}C$ values. Error bars = standard deviation. n = 3 replicates.




B
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-12.00
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-20.00
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δ 13C
Cane - Beet Diet
Beet - Beet Diet
Control (UAS-diap)
Control (Lsp2-Gal4)
Cell death blocked (UAS-diap; Lsp2-Gal4)
Control (UAS-diap)
Control (Lsp2-Gal4)
Cell death blocked (UAS-diap; Lsp2-Gal4)
1
2
3
4
7
Days

value of -19.9 $\pm$ 0.33, characteristic of beet sugar (Fig. 10B). In contrast, ovaries from day-old females from the parental lines that were switched from a cane-sugar to a beet-sugar diet (cane:beet) initially had a cane sugar-like carbon isotope ratio of $\delta^{13}C$= -12.8 $\pm$ 0.25 (Fig. 10B) which shifted as females fed on the beet-sugar diet. The ovaries gradually acquired a carbon isotope ratio corresponding to beet sugar. After day 4 ovaries had a beet sugar signature of $\delta^{13}C$= - 20.9 $\pm$ 0.18 (Fig. 10B), thereby indicating that after 4 days only adult-derived nutrients were contributing to the ovaries. In contrast, in the F1 females where programmed cell death is blocked in the fat cells, the ovaries more rapidly acquired an adult-diet carbon isotope ratio as measured on day 2 (Fig. 10B). These data suggest that fewer larval-derived nutrients are taken up by the ovaries of animals in the absence of fat cell death. In the reciprocal diet experiment (beet:cane), similar result were observed (Fig. 10A). We suggest that as a consequence of inhibiting programmed cell death, larval nutrients are not readily available for uptake by the ovaries, and ovary development is compromised because adult feeding can not compensate for this loss. However, because the elemental analysis provides only a ratio of ^{13}C to ^{12}C, it is not possible to distinguish between a decrease in the uptake of larval-derived nutrients or a compensatory increase in the uptake in adult-derived nutrients. To address this problem we calculated for 2 day-old ovaries the relative amount of carbon derived from the larval-diet based on the $\delta^{13}C$ value and the weight of the ovaries. At day 2, larval-derived nutrients represented ~42% of the carbon present in the ovaries; however, in the F1 females in the absence of larval fat cell death, the larval contribution to the ovaries dropped to 26% (Table 1). These data suggest that cell death of the larval fat cells allows for the rapid transfer of larval nutrients to the ovaries.

Larval Energy Stores are Used for the Maturation of the Ovaries

To determine whether the difference in ovary size affects fecundity we established when the parents (*UAS-diap* or *Lsp2-Gal4)* and F1 females initiated egg laying and measured egg laying capacity. Females were collected upon eclosion (0-2hr), placed into individual wells containing grape agar and monitored for egg laying. Parents began to lay eggs by day 2, while the F1 females were delayed until day 3 post-eclosion (Fig. 11). Females were monitored for seven days for deposition of eggs. The parental females laid ~40 eggs per female while the F1 females laid ~18 eggs per female, a 55% reduction in egg laying capacity (Fig. 12). These data demonstrate that programmed cell death of the larval fat cells drives the rapid development of the ovaries and affects both the initiation of egg laying and egg laying capacity.

Virgin females lay only a limited number of eggs as illustrated in Fig. 11. It is possible that in mated females with their longer egg laying period and higher egg laying capacity, that the F1 females might recover and exhibit normal fecundity. Parental and F1 females were collected upon eclosion (0-2hr) and placed in the individual egg laying wells with two males. Both initial egg laying and egg laying capacity were determined over a 10 day period. In mated females, egg lay commenced by day 2 post-eclosion for both the parental and the F1 animals (Fig. 13), but the overall fecundity of the F1 females was lower. F1 mated females produced only 197 $\pm$ 3 eggs in the first 10 days of adult life, compared to 297 $\pm$ 4 and 307 $\pm$ 4 eggs for the parental females (Fig.14). The rate of egg laying was reduced in the F1 females but approached parental levels during the latter half of the egg laying period (days 6-10) (Fig. 13 and 14). These data taken together suggest

Table 1. At day 2, larval-derived nutrients represent ~ 42% of the carbon present in the ovaries; however, in the F1 females where larval fat cell death is inhibited, the larval contribution to the ovaries drops to 26%. Calculations illustrated below table.

Day 2 - Beet/Cane Diet - Amount of Carbon per Ovary			
	Parental Control (UAS-diap)	**Parental Control (Lsp2-Gal4)**	**Absence of Cell Death**
μg of C from larval diet	**10.1**	**16.2**	**4.7**
μg of C from adult diet	**13.7**	**23.1**	**13.5**
Total μg of C	**23.8**	**39.3**	**18.2**
Percent C from larval diet	**42.4%**	**41.2%**	**25.8%**

The μg of C from the larval or adult diet within the ovaries was calculated according to the following functions:

μg of C = (weight of sample (μg)) x (% total C in sample)

The $\delta^{13}C_{\text{cane sugar}} = -11.25$ and $\delta^{13}C_{\text{beet sugar}} = -23.50$; thus the difference between the range of values is (-23.50) – (-11.25) = -12.25.

μg of C from larval diet = $-[(-23.50 - \delta^{13}C_{\text{ovary}}) / 12.25]$

μg of C from adult diet = $-[(-11.25 - \delta^{13}C_{\text{ovary}}) / 12.25]$

The percent C from the larval diet was calculated according to the following functions:

μg of C from larval diet + μg of C from adult diet = Total μg of C.

μg of C from larval diet / Total μg of C = Percent C from larval diet.

Figure 11. Absence of larval fat-cell death in virgin females leads to a 2-day delay in initiating egg laying. Ectopic expression of the *Drosophila* inhibitor of apoptosis 1 (DIAP1) protein in the larval fat cells blocks the normal cell death of larval fat cells in the adult (Aguila et al., 2007). In the absence of cell death in the larval fat cells, unmated fed females (*Lsp2-GAL4/UAS-diap)* egg laying is delayed to day 3 while in the fed parental controls *(Lsp2-GAL4* and *UAS-diap*) egg laying commences by day 2. Error bars = standard deviation. n= 4 trials with 12 females for each genotype.

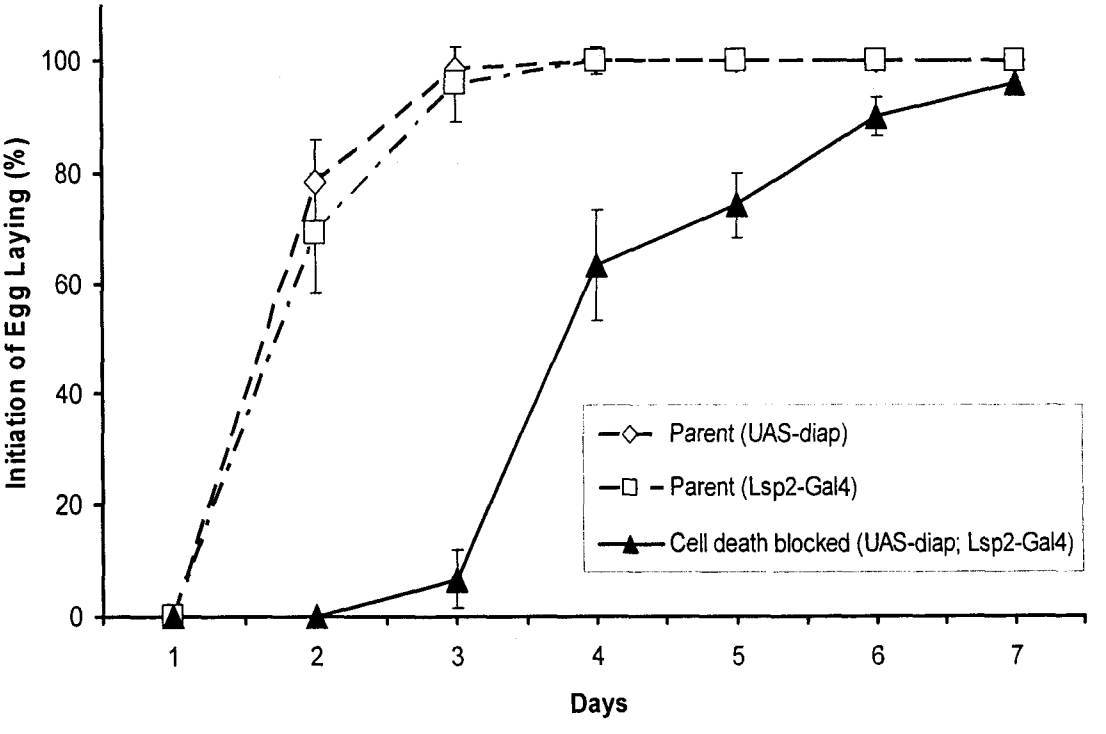
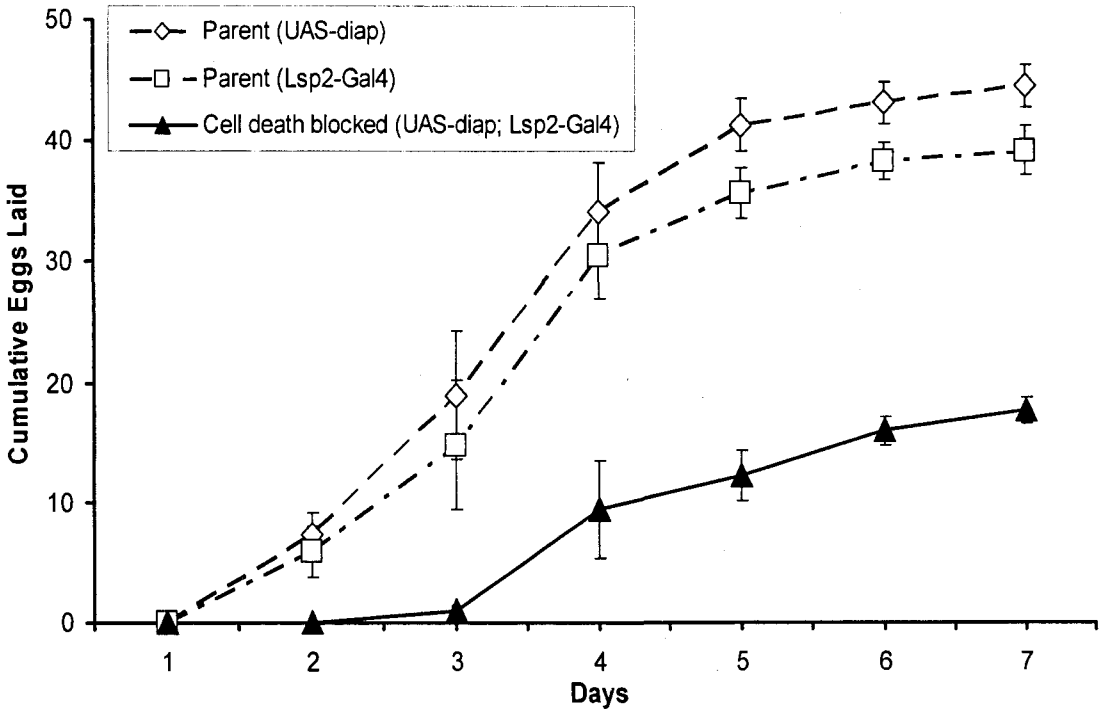


Figure 12. Absence of larval fat-cell death in virgin females leads to a 58% decrease in egg laying capacity. In the absence of cell death in the larval fat cells, fed females (*Lsp2-GAL4/UAS-diap)* egg laying capacity is reduced to 17 +1.3 eggs compared to 37 + 1.4 and 43 + 1.4 eggs for the fed parental controls, *Lsp2-GAL4* and *UAS-diap,* respectively. Error bars = standard deviation. n= 4 trials with 12 females of each genotype per trial.



that the programmed cell death of the larval fat cells triggers the quick release of larval nutrient stores and allows for the rapid maturation of the ovaries necessary for egg laying.

Larval Fat Cell Triacylglycerides Fuels the Maturation of the Ovaries

A possible explanation for the reduced fecundity of the F1 females is that different amounts of energy stores are brought forward into the adult compared to parental controls. We have previously demonstrated that the number of fat cells present in the newly-eclosed female is unaffected by the inhibition of fat-cell death (Aguila et al., 2007). Here, we determine the triglyceride, glycogen, and protein content of F1 and parental females at eclosion and at one day intervals. Upon eclosion, the F1 and parental females exhibit the same triglyceride, glycogen and protein levels. Thus, there is no difference in the energy content between the F1 and parental females at the beginning of adult life. The levels of triglyceride, glycogen and protein remained similar between the F1 and parentals throughout the first 10 days of adult life. The only significant difference was at day 1 where triglyceride levels in the F1 females increased to 94.6 ± 3.1 μg per female which was over 33% higher than in the parental females (Fig. 15).

Discussion

The life cycle of *Drosophila melanogaster* is distinguished by feeding and non-feeding periods that are closely linked to unique developmental stages. Energy reserves acquired during the larval stage are stored in the larval fat body to fuel the later re-architecture of the animal to the adult form during metamorphosis. These larval nutrient stores are transferred to the adult in the dissociated cells of the larval fat body. In the

Figure 13. In the absence of larval fat-cell death mated females lay fewer eggs. In the absence of cell death in the larval fat cells, mated females exhibit a delay in egg laying and an overall reduction in egg laying capacity . In mated females, egg lay commenced by day 2 post-eclosion for all animals, but total fecundity over ten days decreased by 35% for experimental versus control animals. In the absence of cell death in the larval fat cells, mated females (*Lsp2-GAL4/UAS-diap)* laid 197 $\pm$ 3.1 eggs in the first 10 days of adult life, compared to 297 $\pm$ 4.1 and 307 $\pm$ 3.9 eggs for controls. Error bars = standard deviation. n = 4 trials with 12 females for each genotype per trial.

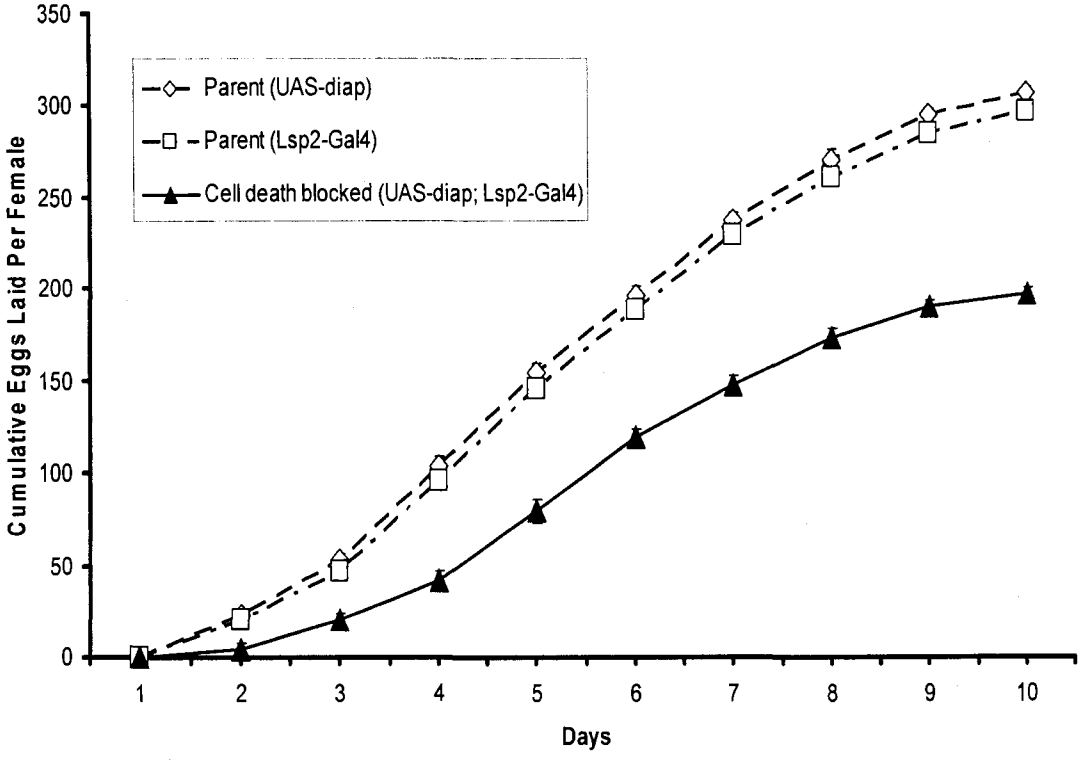


Figure 14. In the absence of larval fat-cell death mated females cumulatively lay fewer eggs. In the absence of cell death in the larval fat cells, mated females exhibit a delay in egg laying and an overall reduction in egg laying capacity. Error bars = standard deviation. n = 4 trials with 12 females per trial for each genotype.

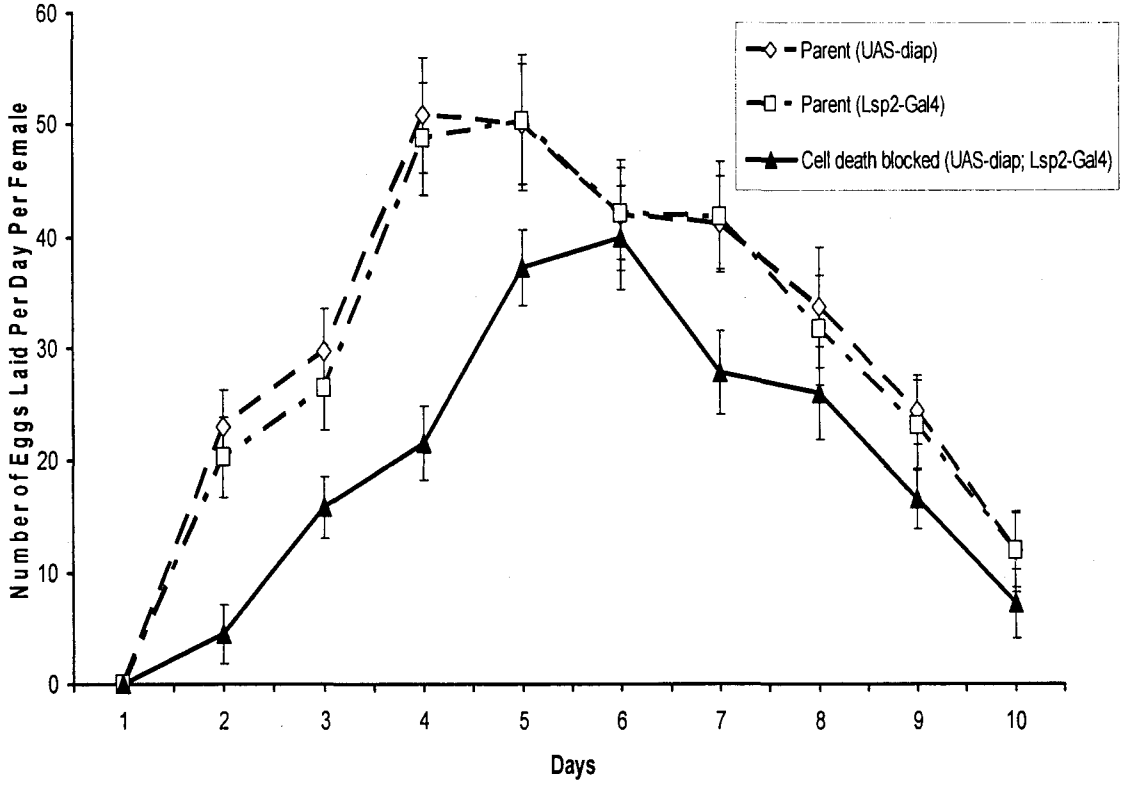
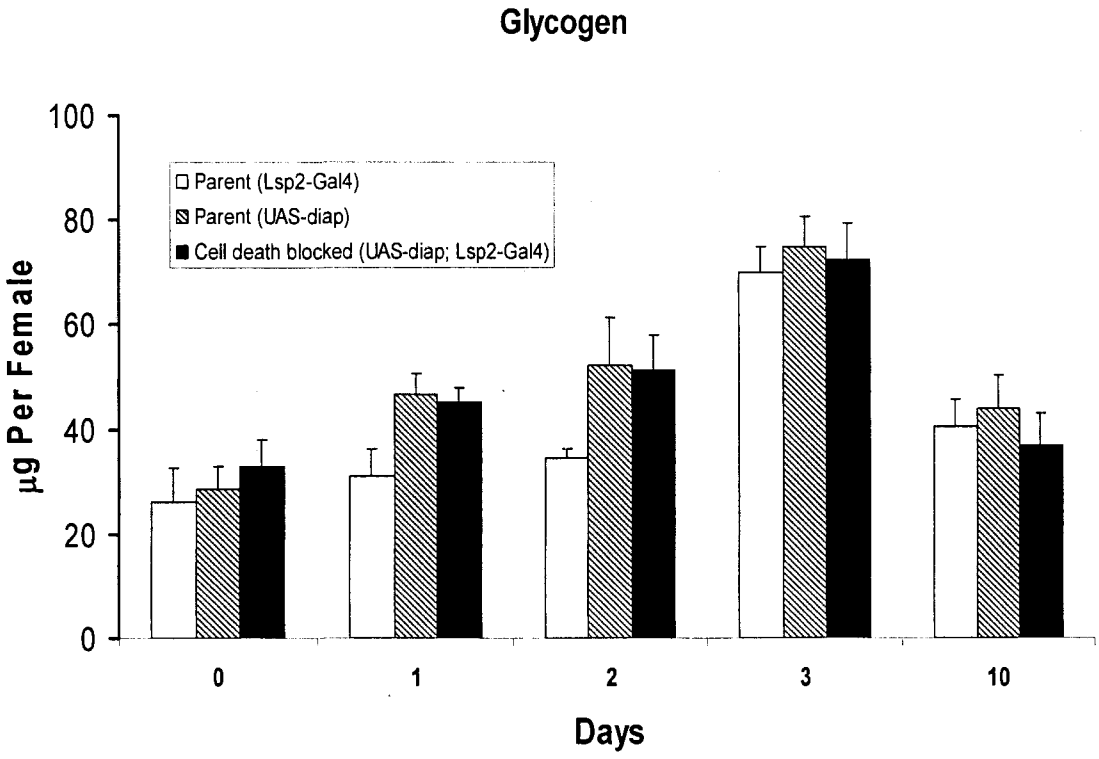
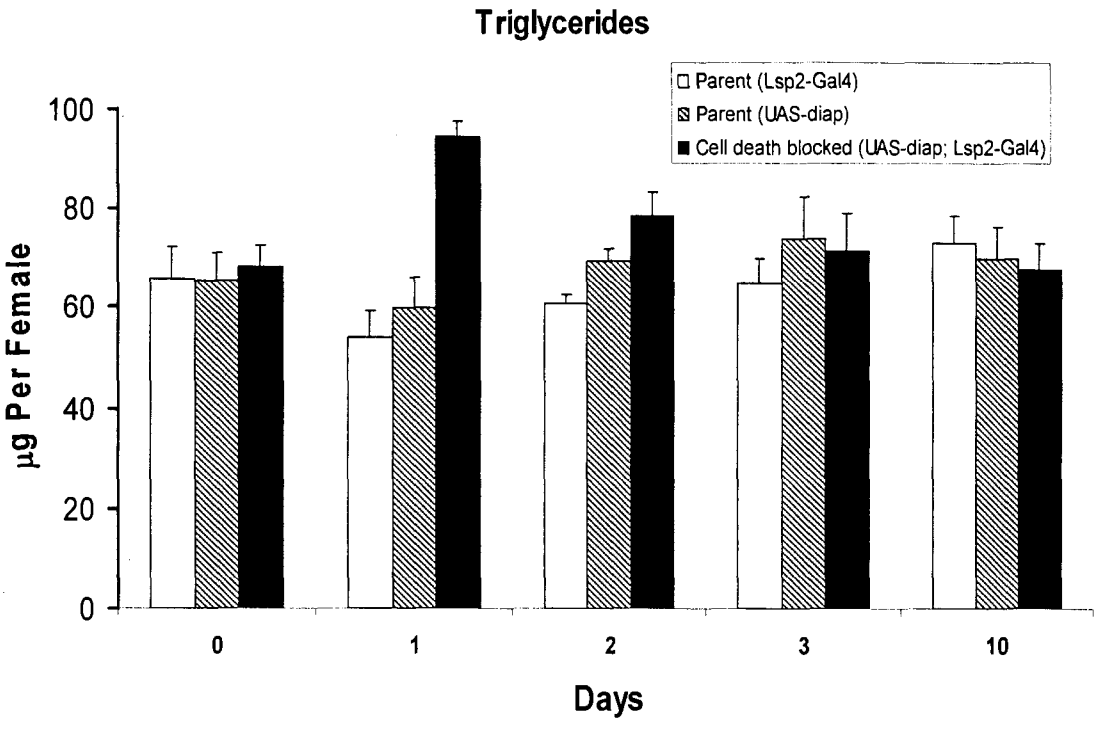
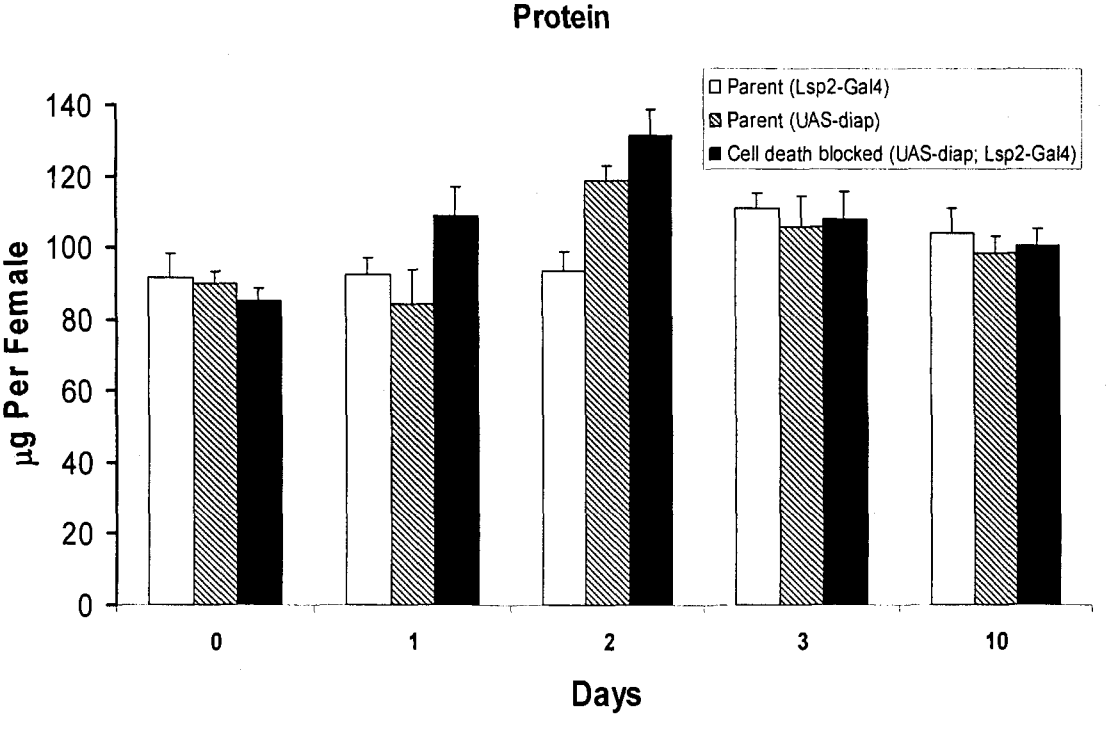


Figure 15. Absence of larval fat-cell death in virgin females leads to a normal energy budget except for a transient accumulation of triglycerides at day 1. In the absence of cell death in the larval fat cells, unmated females (*Lsp2-GAL4/UAS-diap)* the energy reserves brought forward through pupal development remain unchanged from the reserves of the control parents (NE, newly eclosed). At day 1, in the absence of cell death in the larval fat cells, females exhibit a transient accumulation of triglycerides not seen in the parental lines. *Lsp2-GAL4,* parental control, *UAS-diap,* parental control, and *Lsp2-GAL4/UAS-diap,* fat cell death inhibited. Error bars = standard deviation. n= 24 for NE, 2-day, and 3-day; 20 for 10-day.




Triglycerides
Parent (Lsp2-Gal4)
Parent (UAS-diap)
Cell death blocked (UAS-diap; Lsp2-Gal4)
µg Per Female
100
80
60
40
20
0
0
1
2
3
10
Days


Protein
Parent (Lsp2-Gal4)
Parent (UAS-diap)
Cell death blocked (UAS-diap; Lsp2-Gal4)
µg Per Female
140
120
100
80
60
40
20
0
0
1
2
3
10
Days

adult, the larval nutrients are presumably allocated to support the maturation of somatic and gonadal tissues.

In females, the gonads develop rapidly during the first 24-48 hours post eclosion. We hypothesized that gonadal tissue growth depends on the energy stores brought forward by the larval fat cells and that these stores must be readily available during this crucial time period for ovary development. During this important time, the normal cell death of larval fat cells leads to the transfer of energy stores to the ovaries. In contrast, if cell death of larval fat cells is inhibited, then the energy stores would not be available for gonadal development.

In the absence of cell-death animals, we demonstrated that the proper development of the ovaries is delayed and overall fecundity is reduced. For these absence of cell death experimental animals, two different situations could be envisioned to explain the results. On one hand, the larval energy stores may be mobilized to support the soma at the expense of proper gonadal development. Because these animals would allocate less of their larval energy stores to ovary development, the nutrients may be available to support the soma and thus lead to an increase in starvation resistance (Aguila et al. 2007). Moreover, the decrease in available energy sources would lead to decreases in gonadal growth and lower fecundity in cell-death inhibited females. On the other hand, the larval energy stores may not be mobilized to any tissue at all. To investigate these varying situations, we utilized stable isotope techniques and were able to track whether carbon in the ovaries was derived from the larval fat body or adult feeding.

The Importance of Larval Energy Stores for the Development of the Ovaries

Previous studies have shown the importance of the larval fat cells for successful survival through metamorphosis. Along with producing small amounts of growth factors, the larval fat body contains large stores of proteins, carbohydrates, and lipids. These nutrients are utilized during metamorphosis and shortly after eclosion, but if an animal is starved, they are precociously mobilized into the hemolymph to support the animal during starvation (Britton et al., 2002). In our study, we used stable carbon isotopes to show that energy from the larval fat cells is mobilized to the ovaries. We were able to manipulate the carbon signature of the gonadal tissue by providing diets that differed in carbon isotope ratios, and by blocking the normal cell death of the larval fat body. In animals that had their larval fat cell death blocked, ovaries acquired an adult isotope signature more quickly.

In *Drosophila*, dietary sugar is very important for successful ovary development and egg production. Ovaries from animals on the cane:beet diet had a strong cane isotope signature at day 1 (Fig. 10B). On the other hand, on the beet:cane diet, the ovaries from animals at day 1 had a less complete beet signature (Fig. 10A). This is likely due to the fact that some carbon was derived from the corn meal and yeast in the diet. Corn, like sugar cane, is a C4 plant; therefore, corn meal (and yeast, if grown on a C4 carbon source) consumed during larval feeding in the first 24 hours of adult life may have affected the carbon isotope signature. At every age, it has been shown that half of the carbon in eggs is derived from dietary sugar (Min et al., 2006); the rest must be derived from other sources. Since all the diets contained the same amounts of corn meal and yeast and only differed in the type of sugar, the effects would be the same across all diets. For

both control and cell-death inhibited experimental animals that were raised on the same diet as larvae and adults (cane:cane or beet:beet), the isotope ratios remained consistent 7 days post-eclosion. Females on a cane:cane diet attained isotope signatures of approximately -12.4 $\pm$ 0.29 and those on a beet:beet diet had a signature of approximately -19.9 $\pm$ 0.33.

In contrast, animals that were raised on one diet as larvae and switched to the other as adults showed a very different pattern for isotope signatures. In both the cane:beet and beet:cane group, the isotope signature of the cell-death inhibited animals migrated towards an adult carbon source more quickly than did that of control animals. At days 1 to 3 post-eclosion, the cell-death inhibited animals consistently contained carbon signatures closer to the adult signature (Fig. 10A & 10B). Furthermore, isotope signatures converged at 4 days after eclosion in experimental and control animals. In cell-death inhibited animals, larval fat cell death is completed by 90-96 hours post-eclosion (Aguila et al., 2007); therefore, by day 4, the larval fat reserves would have become available to all animals. Taken these data together, it can be concluded that cell-death inhibited animals utilize a larger ratio of adult energy stores earlier in adulthood (days 1 to 3 post-eclosion) compared to control animals.

In cell-death inhibited virgin females, it is important to note the possibility of compensatory feeding of the animal as an adult to offset the lack of availability of energy stores from the larval fat cells. Compensatory feeding may or may not lead to a relevant change in overall ovary development. In the end, the mass of the cell-death inhibited animals' ovaries was lower and the ovaries were visibly much smaller from days 1 to 3 post-ecolosion. (Fig. 9).

Effects of Blocking Normal Cell Death of the Larval Fat Cells on Reproductive Success

Normally, wild-type females contain fully developed eggs ready to be laid approximately two days after eclosion. In cell-death inhibited virgin females, initiation of egg laying was delayed and overall egg laying capacity decreased compared to controls (Fig. 11 and 12). It is important to note that the virgin female pattern of egg laying is quite different from that of mated females (Bouletreau-Merle, 1971). Mated females can hypothetically lay 750-1000 eggs in a perfect environment (Gowen and Johnson, 1946), but fecundity is extremely sensitive to environmental conditions including humidity, temperature (Siddiqui and Barlow, 1972), population density (Pearl, 1932), and the availability of food, most importantly yeast (David et al., 1971; Simmons and Bradley, 1997). Furthermore, the mere presence of males increases the fecundity of females even if fertilization does not occur. In fact, egg laying is stimulated by the paragonial secretions of males, even if they are sterile (Hihara, 1981). In mated females, egg lay commenced by day 2 post-eclosion for all animals, but total fecundity over ten days decreased by 35% in experimental versus control animals. Cell-death inhibited animals laid fewer eggs than control animals from days 2-4, yet began to lay wild-type levels of eggs at days 5-6. (Fig. 13 and 14). This demonstrated that while mated experimental animals eventually were able to lay wild-type levels of eggs on a per day basis, the cumulative number of eggs laid over a ten day period never reached wild-type levels.

The Importance of Larval Fat Cell Triacylglycerides for Ovary Maturation

We demonstrated that cell-death inhibited animals did not differ in the amounts of lipids, carbohydrates, or proteins at eclosion compared to control animals (Fig. 15). This important finding leads us to conclude that cell-death inhibited animals' increase in

starvation resistance and decrease in fecundity was not caused by differences in initial energy stores. In cell-death inhibited animals, energy stores from the larval fat body are not properly transferred to the ovaries. Furthermore, we demonstrated that cell-death inhibited animals accumulate more lipids compared to controls 1 day post eclosion. Lipids contain two times more energy than carbohydrates or proteins and thus would be a more efficient energy source for a stressed organism. Therefore, storing high amounts of lipids may be beneficial for animals that are in a starvation environment or those who are unable to properly transfer the stored nutrients from the larval fat cells to tissues in the early adult. These stores may serve as an effective nutrient reservoir that the animal may be able to utilize as it matures into adulthood.

In the absence of cell death in the larval fat cells, one-day old females (*Lsp2-GAL4/UAS-diap*) had 94.6 + 3.1 μg triglycerides compared to 53.9 ± 3.7 and 59.9 ± 6.1 μg for the control genotypes, *Lsp2-GAL4* and *UAS-diap*, respectively (Fig. 15). This was a significant finding because it may be correlated to the noticeable delay in egg laying as well as reduced fecundity for cell-death inhibited females compared to controls in virgins and mated females. The amount of lipids at day 1 and 2 may affect the fecundity and oviposition of experimental animals, in addition to starvation resistance (Aguila et al. 2007). This may indicate compensatory feeding by cell-death inhibited animals as adults, leading to extra lipid stores. In early adulthood, these animals may not be able to efficiently allocate the necessary amounts of energy stores to both the soma and developing ovaries, and the organism may enter a starvation-like response. Previous studies have demonstrated that selecting *Drosophila* for starvation resistance changes the timing of egg production in these animals. Selected starvation animals produce fewer

eggs early in adulthood compared to controls, but later these animals have higher ovariole numbers (Wayne et al., 2006). Blocking larval fat cell death in animals may emulate the effects seen in starvation selected animals. Therefore, it would be more beneficial for the animal to store more lipids in order to support the somatic tissues during this time.

This experiment supports the idea that a critical period exists in early adulthood of female flies to mobilize nutrient stores from the larval fat body to the ovaries for proper gonadal development. If this "window of opportunity" is disrupted, then overall gonadal development is compromised. It is important to recall that cell-death inhibited animals eventually lose their larval fat cells, but at a much slower rate than control animals. For proper ovarian development, there may be a specific time when energy stores are necessary. If energy stores are not mobilized to the gonadal tissue, then the ovaries may develop at a slower pace and overall reproductive success will decline. In the absence of cell death in the larval fat cells, the capacity of these animals to lay eggs decreases and is delayed. Yet adult feeding 5-6 days post eclosion may compensate for these nutrients and allow animals to begin to lay wild-type levels of eggs.

Conclusion

Environmental stress, such as starvation, could affect a tradeoff between reproduction and survival, but the physiological mechanisms underlying environmental mediation of the tradeoff are largely unknown. In our earlier study, we showed a fundamental role for the larval fat cells in adult starvation resistance (Aguila et al., 2007). Furthermore, we demonstrated that blocking death of the larval fat cells in the adult increased starvation resistance. We speculate that death of the larval fat cells allows bulk

recycling of cellular components and release of the energy stores for utilization by the adult. This presented a paradox in that while animals utilize energy stores from larval fat cells undergoing cell death to combat starvation, their resistance to starvation is increased when cell death is inhibited. In this study, we investigated the possibility that in young adults, larval fat-cell energy stores are normally allocated to the gonads. We suggest that when normal larval fat cell death is blocked, energy stores of the fat cells are available during starvation, whereas energy that has entered the gonads is not.

Our current findings shed some new light upon the mechanism of energy allocation from the larval fat cells to the adult somatic and reproductive tissues. By using stable isotope analysis, we were able to specifically track larval- versus adult-derived nutrients in the ovaries. We also demonstrated the importance for the adult animal to have readily available access to nutrient stores acquired by the larva for proper ovarian development and fecundity.

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CHAPTER 5

CONCLUSION

In this dissertation, I have demonstrated the importance and a role of the larval fat cells in the adult *Drosophila melanogaster* female. It is clear that the larval fat cells serve as a nutritional reservoir for the young adult prior to feeding. Moreover, the energy contents of the larval fat cells may function as a "meal-ready-to-eat" if the adult ecloses in a stressful environment where sufficient food is unavailable. Finally, I demonstrate that the energy stores within the larval fat cells are directly transferred to the adult ovaries and are necessary for proper ovarian development, age of first reproduction, and levels of fecundity.

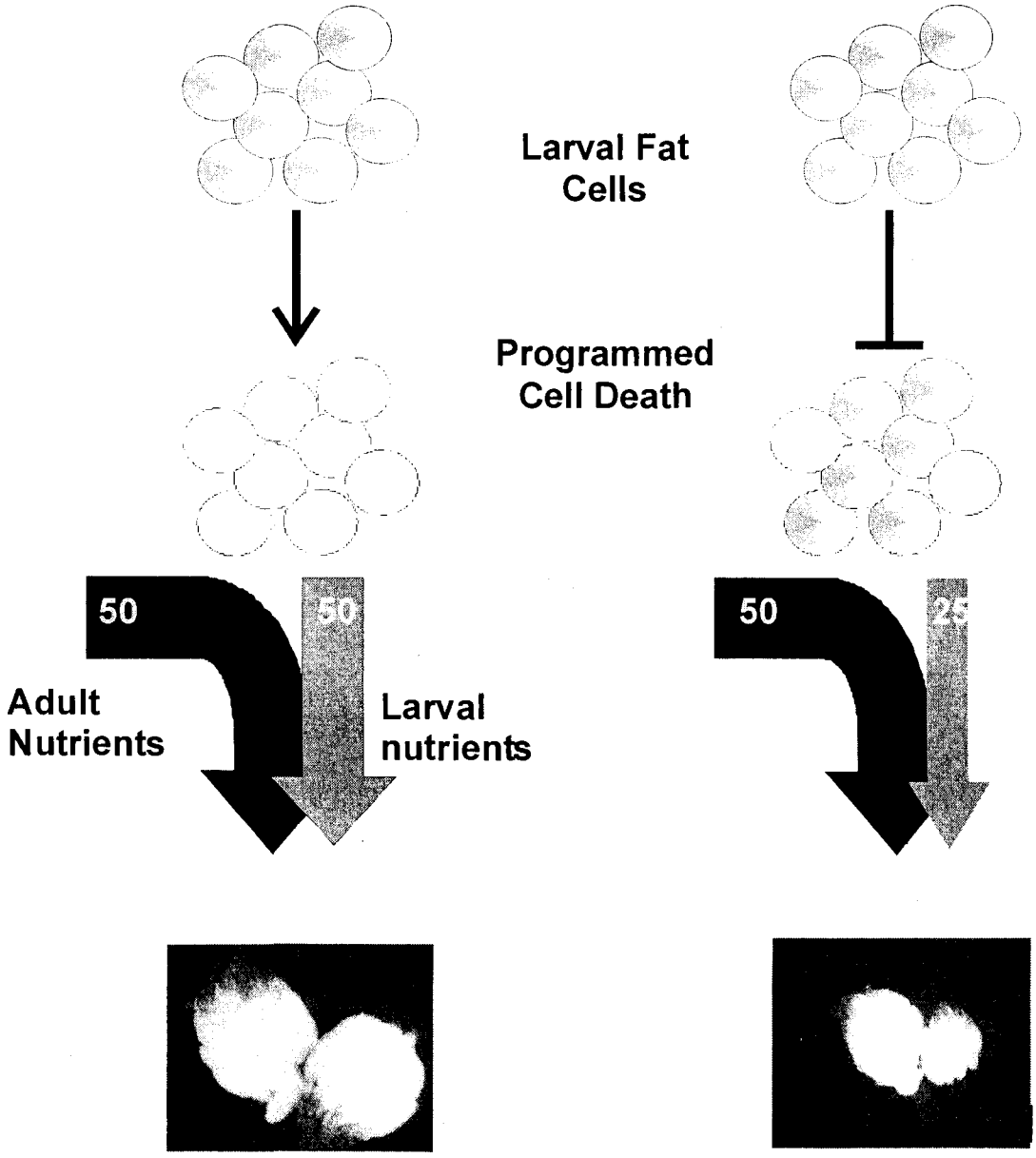
The importance of the larval fat cells for overall adult fitness is clearly observed in insects that do not feed as adults, including the silkworm and mayfly. These animals must acquire all their nutrients during the larval period and must then utilize a mechanism to allocate them throughout the pupal and adult stages. A likely mechanism for energy store utilization in these animals is programmed cell death (PCD) in which larval fat cells release their energy contents for the animals use. In *D. melanogaster*, an interesting question is what type of programmed cell death do larval fat cells undergo in order to transfer their energy contents to adult tissues such as the ovaries. New studies in the *Drosophila* salivary gland, have suggested that multiple degradation pathways act synergistically to break down cells (Berry and Baehrecke, 2007). Furthermore, it has

been shown that various caspase-dependent and –independent factors are required in both autophagic and apoptotic cell death (Berry and Baehrecke, 2007).

Because I ectopically expressed the Drosophila inhibitor of apoptosis (Diap1) in the larval fat body, my model suggests that caspase-induced programmed cell death of the larval fat cells is part of the mechanism leading to proper development of the ovaries (Fig. 16). In wild-type animals that undergo larval fat cell death, larval derived energy stores are transferred efficiently to the ovaries. On the other hand, when programmed cell death of the larval fat cells is inhibited, a portion of the energy stores are unavailable for transfer to the ovaries causing them to be underdeveloped (Fig. 16). With the current findings that components of both the autophagic and apoptotic pathways may be shared, ovarian development might be controlled by parallel PCD pathways of the larval fat cells that could contribute to ovarian growth. In other words, even if a specific *Drosophila* inhibitor of apoptosis is utilized to block the caspase cascade leading to cell death, there still might be another active autophagic pathway that enables stressed cells to undergo PCD and release their energy contents for use by the animal. This alternative pathway may be correlated to both an increase in starvation resistance or a comparable ovary size in animals that have their caspase induced programmed cell death inhibited. This possible explanation may also serve as a foundation for utilizing the larval fat body as a model for investigating the intricacies of programmed cell death.

Future directions for these studies include using the GAL4-UAS system to genetically construct an animal that ecloses as an adult with a considerably depleted levels of larval fat cells. This could serve as an important tool to further investigate the importance of the larval fat cells in starvation resistance and adult reproduction. We

Figure 16. Model for the transfer of larval energy stores to the ovaries. In caspase-induced programmed cell death of the larval fat cells, larval-derived energy stores are transferred as well as adult-derived nutrients from feeding to support ovarian development (left). When programmed cell death of the larval fat cells is inhibited, half of the larval energy stores are no longer found in the ovaries causing them to be underdeveloped (right). This model is supported by the data in figures 9, 10, and 11 and table 1 of this dissertation. Ovaries pictured were dissected 2 days post eclosion.



hypothesize that these animals would succumb to starvation more quickly than control animals. Furthermore, we believe these animals would show a more severe negative effect on adult reproduction. In addition, fecundity assays could be conducted during the entire lifetime of the larval fat cell death blocked mated adult females, in order to observe if they would ever lay wild-type levels of eggs. While I planned on carrying out this experiment, the data from the 10-day fecundity assays demonstrated a gradual plateau of egg laying from days 7 to 10 with the experimental group still laying less eggs compared to the controls (Fig. 13). Finally, cell staining at various pupal and adult stages could be carried out to monitor for either necrosis or programmed cell death. This would allow for a better temporal and spatial understanding of what is occurring to the cells at the cellular level. The results reported in this dissertation as well as the proposed future studies may shed new light upon the diverse mechanisms of programmed cell death and may also lead to new insight regarding the transfer of larval energy stores to the adult.

REFERENCES

Aguila, J.R., Suszko, J., Gibbs, A.G., and Hoshizaki, D.K. (2007). The role of larval fat cells in adult *Drosophila melanogaster*. *J. Exp. Biol.* **210**, 956-63.

Andres, A., Shreck, J., Boyles, R., Bond, N., Hoshizaki, D. and Merriam, J. (2004). Dissecting salivary gland secretion using a gene-trap strategy. 45th Annual Drosophila. Research Conference, 915C.

Arends, M.J., Morris, R.G., and Wyllie, A.H. (1990). Apoptosis: The role of the endonuclease. *Am. J. Pathol.* **136**, 593-608.

Bainbridge, S. P. and Bownes, M. (1981). Staging the metamorphosis of Drosophila melanogaster. *J. Embryol. Exp. Morphol.* **66**, 57-80.

Baldal, E. A., van der Linde, K., van Alphen, J. J. M., Brakefield, P. M. and Zwaan, B. J. (2004). The effects of larval density on adult lifehistory traits in three species of Drosophila. *Mech. Ageing Dev.* **126**, 407-416.

Berry, D.L. and Baehrecke, E.H. (2007). Growth arrest and autophagy are required for salivary gland cell degradation in *Drosophila*. *Cell* **131**, 1137-48.

Bodenstein, D. (1950). The postembryonic development of Drosophila. In *Biology of Drosophila* (ed. M. Demerec), pp. 275-375. Cold Spring Harbor:Cold Spring Harbor Laboratory Press.

Boggs, C.L. and Freeman, K.D. (2005). Larval food limitation in butterflies: effects on adult resource allocation and fitness. *Oecologia* **3**, 353-61.

Bortner, C.D. and Cidlowski, J.A. (2002). Apoptotic volume decrease and the incredible shrinking cell. *Cell Death Differ.* **9**, 1307-1310.

Bouletreau-Merle, J. (1971). Influences genetiques sur la retention des oeufs chez les femelles vierges de Drosophila melanogaster et le nombre d'oeufs formes a partir des reserves larvaires. *Ann. Zool. Ecol. Anim.* **3**, 481-492.

Brand, A. H. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415.

Britton, J.S., Lockwood, W.K., Li., L., Cohen, S.M., and Edgar, B.A. (2002). Drosophila's insulin/PI3-kinase pathway coordinates cellular metabolism with nutritional conditions. *Dev. Cell.* **2**, 239-49.

Butterworth, F. M. (1972). Adipose tissue of Drosophila melanogaster. V. Genetic and experimental studies of an extrinsic influence on the rate of cell death in the larval fat body. *Dev. Biol.* **28**, 311-325.

Chiang, C. H. (1963). Tactic reactions of young adults of *Drosophila melanogaster. Am. Midl. Nat.* **70**, 329-338.

Chiang, H.C., and Hodson, A.C. (1950). An analytical study of population growth in *Drosophila melanogaster. Ecol. Monographs* **20**, 173-206.

Chippindale, A. K., Chu, T. J. F. and Rose, M. R. (1996). Complex tradeoffs and the evolution of starvation resistance in *Drosophila melanogaster. Evolution* **50**, 753-766.

Chippindale, A. K., Gibbs, A. G., Sheik, M., Yee, K. J., Djawdan, M., Bradley, T. J. and Rose, M. R. (1998). Resource allocation and the evolution of desiccation

resistance in laboratory-selected *Drosophila melanogaster*. *Evolution* **52**, 1342-1352.

Church, R. B. and Robertson, F. W. (1966). Biochemical analysis of genetic differences in the growth of Drosophila. *Genet. Res.* **7**, 383-407.

Colombani, J., Raisin, S., Pantalacci, S., Radimerski, T., Montagne, J. and Leopold, P. (2003). A nutrient sensor mechanism controls *Drosophila* growth. *Cell* **114**, 739-49.

Dai, J. D. and Gilbert, L. I. (1997). Programmed cell death of the prothoracic glands of *Manduca sexta* during pupal-adult metamorphosis. *Insect Biochem. Mol. Biol.* **27**, 69-78.

David, J.R., Fouillet, P., and Arens, M.F. (1971). Influence repulsive de la levure vivante sur l'oviposition de la Drosophile: Importance de la salissure progressive des cages et des differences innees entre les femelles. *Rev. Comp. Animal* **5**, 277-288.

David, J., and Merle, J. (1968). A re-evaluation of the duration of egg chamber stages in oogenesis of *Drosophila melanogaster*. *Drosophila Inform. Serv.* **43**, 122-123.

Drosophila Ovary. (2006). [biolpc22.york.ac.uk/drosophila/ovary/index.html].

Edgecomb, R.S., Harth, C.E. and Schneiderman, A.M. (1994). Regulation of feeding behavior in adult Drosophila melanogaster varies with feeding regime and nutritional state. *J. Exp. Biol.* **197**, 215-235.

Feder, M. E., Blair, N. and Figueras, H. (1997). Natural thermal stress and heat-shock protein expression in Drosophila larvae and pupae. *Funct. Ecol.* **11**, 90-100.

Fischer, D., O'Brien, D.M., and Boggs, C.L. (2004). Allocation of larval and adult resources to reproduction in a fruit-feeding butterfly. *Functional Ecology* **18**, 656-663.

Gefen, E., Marlon, A. J. and Gibbs, A. G. (2006). Selection for desiccation resistance in adult *Drosophila melanogaster* affects larval development and metabolite accumulation. *J. Exp. Biol.* **209**, 3293-300.

Gibbs, A. G., Perkins, M. C. and Markow, T. A. (2003). No place to hide: microclimates of Sonoran Desert Drosophila. *J. Therm. Biol.* **28**, 353-362.

Goldsmith, M.R., Shimada, T. and Hiroaki, A. (2004). The genetics and genomics of the silkworm, *Bombyx mori. Annu. Rev. Entomol.* **50**, 71-100.

Gowen, J.W. and Johnson, L.E. (1946). On the mechansism of heterosis. I. Metabolic capacity of different races of Drosophila melanogaster for egg production. *Am. Nat.* **80**, 149-179.

Goyal, L. (2001). Cell death inhibition: Keeping caspases in check. *Cell* **104,** 805-808.

Goyal, L., MacCall, K., Agapite, J., Hartwieg, E., and Steller, H. (2000). Induction of apoptosis by *Drosophila reaper, hid* and *grim* through inhibition of IAPfunction. *EMBO J.* **19**, 589-597.

Hafen, E. (2004). Interplay between growth factor and nutrient signaling: lessons from *Drosophila* TOR. *Curr. Top. Microbiol. Immunol.* **279**, 153-67.

Hay, B.A. (2000). Understanding IAP function and regulation: A view from *Drosophila. Cell Death Differ.* **7**, 1045-1056.

Hay, B.A., Wassarman, D.A., and Rubin, G.M. (1995). *Drosophila* homologs of baculovirus inhibitor of apoptosis proteins function to block cell death. *Cell* **83,** 1253-1262.

Hihara, F. (1981). Effects of the male accessory gland secretion on oviposition and remating in females of Drosophila melanogaster. *Zool. Sci.* (Tokyo) **90**, 307-316.

Hoffmann, J.A., Reichhart, J.M. and Hentru, C. (1996). Innate immunity in higher insects. *Curr. Opin. Immunol.* **8**, 8-13.

Hoshizaki, D. K. (2005). Fat-cell development. In *Complete Molecular Insect Science.* Vol. 2 (ed. L. I. Gilbert, K. Iatrou and S. Gill), pp. 315-345. Berlin:Elsevier.

Hoshizaki, D. K., Lunz, R., Ghosh, M. and Johnson, W. (1995). Identification of fat cell enhancer activity in *Drosophila melanogaster* using P-element enhancer traps. *Genome* **38**, 497-506.

Jiang, C., Baehrecke, E. H. and Thummel, C. S. (1997). Steroid regulated programmed cell death during Drosophila metamorphosis. *Development* **124**, 4673-4683.

Jochova, J., Quaglino, D., Zakeri, Z., Woo, K., Sikorska, M., Weaver, V. and Lockshin, R. A. (1997). Protein synthesis, DNA degradation, and morphological changes during programmed cell death in labial glands of *Manduca sexta. Dev. Genet.* **21**, 249-257.

Kawamura, K., Shibata, T., Saget, O., Peel, D. and Bryant, P.J. (1999). A new family of growth factors produced by the fat body and active on *Drosophila* imaginal disc cells. *Development* **126**, 211-19.

Kerkis, J. (1933). Development of gonads in hybrids between *Drosophila melanogaster* and *Drosophila simulans. J. Exptl. Zool.* **66**, 477-509.

Kerr, J. F., Wyllie, A. H. and Currie, A. R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* **26**, 239 257.

Klionsky, D.J. and Emr, S.D. (2000). Autophagy as a regulated pathway of cellular degradation. *Science* **290**, 1717-21.

Lee, C. Y. and Baehrecke, E. H. (2001). Steroid regulation of autophagic programmed cell death during development. *Development* **128**, 1443-1455.

Lockshin, R.A. and Zakeri, Z. (2001). Programmed cell death and apoptosis: Origins of the theory. *Nat. Rev. Mol. Cell Biol.* **2**, 545-550.

McCafferty, W.P. (1994). Distributional and classificatory supplement to the burrowing mayflies (*Ephemeroptera: Ephemeroidea)* of the United States. *Entomological News* **105**, 1-13.

Min, K.J., Hogan, M.F., Tatar, M, and O'Brien, D.M. (2006). Resource allocation to reproduction and soma in Drosophila: a stable isotope analysis of carbon from dietary sugar. *J. Insect Physiol.* **52**, 763-770.

Morin, X., Daneman, R., Zavortink, M. and Chia, W. (2001). A protein trap strategy to detect GFP-tagged proteins expressed from their endogenous loci in Drosophila. *Proc. Natl. Acad. Sci. USA* **98**, 15050-15055.

Muro, I., Hay, B.A., and Clem, R.J. (2002). The *Drosophila* DIAP1 protein is required to prevent accumulation of a continuously generated, processed form of the apical caspase Dronc. *J. Biol. Chem.* **277**, 49644-49650.

Nelliot, A., Bond, N. and Hoshizaki, D. K. (2006). Fat-body remodeling in *Drosophila melanogaster*. *Genesis* **44**, 396-400.

O'Brien, D.M., Fogel, M.L., and Boggs, C.L. (2002). Renewable and nonrenewable resources: amino acid turnover and allocation to reproduction in Lepidoptera. *Proc. Natl. Acad. Sci. USA.* **99**, 4413-18.

Pearl, R. 1932. The influence of density of population upon egg production in Drosophila melanogaster. *J. Exp. Zool.* **63**, 57-84.

Postlethwait, J. H. and Jones, G. J. (1978). Endocrine control of larval fat body histolysis in normal and mutant *Drosophila melanogaster. J. Exp. Zool.* **203**, 207-214.

Poulson, D.F. (1950). Histogenesis, organogenesis, and differentiation in the embryo of Drosophila melanogaster Meigen. *In "Biology of Drosophila"* (M. Demerec, ed.), pp. 168-274. Wiley, New York.

Quinn, L.M., Dorstyn, L., Colussi, P.A., Chen, P., Coombe, M., Abrams, J., Kumar, S., and Richardson, H. (2000). An essential role for the caspase Dronc in developmentally programmed cell death in *Drosophila. J. Biol. Chem.* **275**, 40416-40424.

Raguso, R.A., Ojeda-Avila, T., Desai, S., Jurkiewicz, M.A. and Woods, H.A. (2007). The influence of larval diet on adult feeding behaviour in the tobacco hornworm moth, *Manduca sexta. J. Insect Physiol.* **9**, 923-32.

Razik, M.A. and Cidlowski, J.A. (2002). Molecular interplay between ion channels and the regulation of apoptosis. *Biol. Res.* **35**, 203-207.

Richard, D. S., Arnim, A. E. and Gilbert, L. I. (1993). A reappraisal of the hormonal regulation of larval fat body histolysis in female *Drosophila melanogaster. Experientia* **49**, 150-156.

Riddiford, L. M. (1993). Hormones and Drosophila development. In *TheDevelopment of Drosophila*. Vol. 2 (ed. M. Bate and A. M. Arias), pp. 899-939. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Rizki, T. M. (1969). Genetics and evolution of a cell phenotype in Drosophila. *Jap. J. Genet*. **44**, S51-S57.

Robertson, C. W. (1936). The metamorphosis of *Drosophila melanogaster*, including an accurately timed account of the principal morphological changes. *J. Morphol.* **59**, 351-399.

Robertson, F.W., and Sang, J.H. (1944). The ecological determinants of population growth in a *Drosophila* culture. I. Fecundity of adult flies. *Proc. Roy. Soc.* **B132**, 258-277.

Rose, M.R., and Bradley, T.J. (1998). Evolutionary physiology of the cost of reproduction. *Oikos* **83**, 443-451.

Ruiz-Dubreuil, G., Burnet, B., Connolly, K., and Furness, P. (1996). Larval foraging behaviour and competition in *Drosophila melanogaster. Heredity.* **76**, 55-64.

Rusten, T. E., Lindmo, K., Juhasz, G., Sass, M., Seglen, P. O., Brech, A. and Stenmark, H. (2004). Programmed autophagy in the Drosophila fat body is induced by ecdysone through regulation of the PI3K pathway. *Dev. Cell* **7**, 179-192.

Salvesen, G.S. (2002). Caspases: Opening the boxes and interpreting the arrows. *Cell Death Differ.* **9**, 3-5.

Saveliev, V. (1928). On the manifold effect of the gene vestigial in *Drosophila melanogaster. Trav. Soc. Naturalistes Leningrad* **58**, 65-88.

Schmid, D. and Muenz, C. (2007). Innate and adaptive immunity through autophagy. *Immunity*. **27**, 11-21. Review.

Schweichel, J.U. and Merker, H.J. (1973). The morphology of various types of cell death in prenatal tissues. *Teratology* 7, 253-266.

Sevenster, J. G. and Vanalphen, J. J. M. (1993). A life-history trade-off in Drosophila species and community structure in variable environments. *J. Anim. Ecol.* **62**, 720-736.

Siddiqui, W.H. and Barlow, C.A. (1972). Population growth of Drosophila melanogaster (Diptera: Drosophilidae) at constant and alternating temperatures. *Ann. Entomol. Soc. Am.* **65**, 993-1001.

Simmons, F. H. and Bradley, T. J. (1997). An analysis of resource allocation in response to dietary yeast in *Drosophila melanogaster*. *J. Insect Physiol.* **43**, 779-788.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., Klenk, D.C. (1985). Measurement of protein using bicinchoninic acid. *Anal Biochem.* **150**, 76-85.

Sonnenblick, B.P. (1950). The early embryology of Drosophila melanogaster. *In "Biology of Drosophila"* (M. Demerec, ed.), pp. 62-167. Wiley, New York.

Sonnenblick, B.P. (1941). Germ cell movements and sex differentiation of the gonads in the Drosophila embryo. *Proc. Natl. Acad. Sci.* **27,** 1204-1206.

Starr, C. (2006). Biology: Concepts and Applications 6th ed., pp. 102-103. Thomson Brooks/Cole, California.

Suzuki, K., Kostin, S., Person, V., Elsasser, A., and Schaper, J. (2001). Time course of the apoptotic cascade and effects of caspase inhibitors in adult rat ventricular cardiomyocytes. *J. Mol. Cell Cardiol.* **33**, 983-994.

Tu, M.P. and Tatar, M. (2003). Juvenile diet restriction and the aging and reproduction of adult *Drosophila melanogaster*. *Aging Cell* **2**, 327-33.

Van der Merwe, Nikolaas. (1982). Carbon Isotopes, Photosynthesis and Archaeology. *American Scientist* **70,** 596-606.

Wang, S. L., Hawkins, C. J., Yoo, S. J., Muller, H. A. and Hay, B. A. (1999). The Drosophila caspase inhibitor DIAP1 is essential for cell survival and is negatively regulated by HID. *Cell* **98**, 453-463.

Wayne, M.L., Soundararajan, U., and Harshman, L.G. (2006). Environmental stress and reproduction in Drosophila melanogaster: starvation resistance, ovariole numbers and early age egg production. *BMC Evol. Biol.* **6**:57, 1-10.

Webb, S.C., Hedges, R.E.M., and Simpson, S.J. (1998). Diet quality influences the d13C and d15N of locusts and their biochemical components. *J. Exp. Biol.* **201**, 2903-2911.

Werner, R.A., and Brand, W.A. (2001). Referencing strategies and techniques in stable isotope ratio analysis. *Rapid Communications In Mass Spectrometry* **15,** 501-519.

White, K., Grether, M.E., Abrams, J.M., Young, L., Farrell, K., and Steller, H. (1994). Genetic control of programmed cell death in *Drosophila*. *Science* **264,** 677-683.

Wilson, R., Goyal, L., Ditzel, M., Zachariou, A., Baker, D. A., Agapite, J., Steller, H. and Meier, P. (2002). The DIAP1 RING finger mediates ubiquitination of Dronc and is indispensable for regulating apoptosis. *Nat. Cell Biol.* **4**, 445-450.

Wing, J.P., Schwartz, L.M., and Nambu, J.R. (2001). The RHG motifs in *Drosophila* Reaper and Grim are important for their distinct cell death inducing abilities. *Mech. Dev.* **102,** 193-203.

Yang, Y., Fang, S., Jensen, P, Weissman, A.M., and Ashwell, J.D. (2000). Ubiquitin protein ligase activity of IAPs and their degradation in proteosomes in response to apoptotic stimuli. *Science* **288,** 874-877.

Yin, V. P. and Thummel, C. S. (2004). A balance between the diap1 death inhibitor and reaper and hid death inducers controls steroid-triggered cell death in Drosophila. *Proc. Natl. Acad. Sci. USA* **101**, 8022-8027.

Yorimitsu, T. and Klionsky, D.J. (2005). Autophagy: molecular machinery for self eating. *Cell Death and Differentiation* **12**, 1542–1552.

Yoshimori, T. (2004). Autophagy: a regulated bulk degradation process inside cells. *Biochem. Biophys. Res. Commun.* **313**, 453-458.

Yuan, J., Shaham, S., Ledoux, S., Ellis, H.M., and Horvitz, H.R. (1993). The *C. elegans* cell death gene ced-3 encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. *Cell* **75**, 641-652.

Zera, A.J. and Harshman, L.G. (2001). The physiology of life history trade-offs in animals. *Annual Review of Ecology and Systematics* **32**, 95-126.

VITA

Graduate College
University of Nevada, Las Vegas

Jerell Roland Aguila

Home Address:
7971 Limestone Arch Avenue
Las Vegas, Nevada 89178

Degrees:
Bachelor of Science, Biology, 2000
University of Nevada, Reno

Special Honors and Awards:
2004-2007 UNLV GPSA Research Award
2006 Second Place – UNLV GPSA Science and Engineering Platform Presentations
2005 National Science Foundation IDIN Fellowship (NSF EPSCoR Grant EPS0132556 Ring True Two Award)
2005 Advancement to Doctoral Candidacy

Publications:
Aguila, J.R., Suszko, J., Gibbs, A.G., and Hoshizaki, D.K. (2007). The role of larval fat cells in adult *Drosophila melanogaster*. J Exp Biol 210, 956-963.

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Dissertation/Thesis Examination Committee:
Chairperson, Dr. Deborah K. Hoshizaki, Ph. D.
Committee Member, Dr. Allen G. Gibbs, Ph. D.
Committee Member, Dr. Andrew J. Andres, Ph. D.
Committee Member, Dr. Jeffery Shen, Ph. D.
Graduate Faculty Representative, Dr. Ronald K. Gary, Ph. D.