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Investigation of the life history and lipid content consequences of reduced abundance of delta-9 Desaturases in *Drosophila melanogaster*

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Investigation of the life history and lipid content consequences of reduced abundance of delta-9 Desaturases in *Drosophila* melanogaster

By

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Investigation of the life history and lipid content consequences of reduced abundance of delta-9 Desaturases in *Drosophila* melanogaster

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Monounsaturated fatty acids (MUFAs) are essential components in all organisms, and the key enzymes that catalyze the production of MUFA are delta-9 desaturases. These enzymes are ER bound and introduce the first double bonds in saturated fatty acids. To investigate the role that delta-9 desaturases play in *Drosophila* life history and development we performed a series of studies using the RNAi method. Longevity results showed die-off peaks of the knocked-down (KD) flies at day 45. Fecundity study showed decreases in both egg productions and viability of these eggs. Development difficulties were observed in both regular diet and low fat diet. These results suggest disruption of delta-9 desaturase genes have markable impact on not only longevity, but also larval development and offspring production. Development difficulty in these experimental KD groups also supports that desaturases may be specific to fatty acid produced from *de novo* pathways.

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LIST of MULTIMEDIA OBJECTS

LIST of ABBREVIATIONS

SCD : Stearoyl-CoA Desaturase

ER : Endoplasmic Reticulum

Desat 1 : Delta -9 desaturase 1

Desat 2 : Delta -9 desaturase 2

VDRC : Vienna *Drosophila* RNAi Center

UAS : Upstream Activating sequences

KD : Knocked-Down

C16 : Palmitic acid

C16:1 : Palmitoleic acid

C18 : Stearic acid

C18:1 : Oleic acid

C18:2 : Linoleic acid

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Introduction

Delta-9 desaturase, also known as stearoyl-CoA Desaturase (SCD) is an endoplasmic reticulum(ER) bound enzyme that catalyzes monounsaturated fatty acid (MUFA) from saturated fatty acid. MUFAs are essential in all organisms, their high concentration in phospholipids, which are major components for various membranes, contribute greatly for maintaining membrane fluidity. One of the main products that form catalyzing saturated fatty acid to MUFA is oleic acid. This eighteen-carbon-unsaturated fatty acid is the main specie in triglyceride. In humans, oleic acid consist half of total lipid composition in the body.

In mammals there are multiple isoforms of SCD, for example, mice have four isoforms of SCD. Studies showed that disruption of SCD in mice causes severe skin and eye mutation. These abnormalities cannot be rescued by feeding essential fatty acids. (6) However, disruption of a SCD isoform may be beneficial in mice, since studies have shown that loss of SCD-1 function reduced body adiposity and increases insulin sensitivity. This resistance to obesity is true when high fat diets are fed to these animals. (7)

In *Drosophila*, there are two genes for SCD, Desat 1 and Desat 2. These genes were first identified by the Wicker-Thomas group in 1997. They found that the genes are highly similar to desaturases in rats and yeast. These desaturases were identified to have preference on palmitate and myristate as substrates. (11) Recently, support for the relationship between delta-9 desaturase gene and cuticular pheromone were found. (2) Thus, delta-9 desaturase gene also plays an important role in *D*. melanogaster mating behavior. (5)

Previous studies in *C*. elegan shows that animals live longer with nhr-80 mutant compare to nhr-49 mutant, but both live shorter than wild type. Nhr-80 and nhr-49 are both regulator of lipids, which are required for desaturase functions. When these genes are mutated, overall lipid content decreases and some mutant strains show markable decrease in targeted fatty acids compared to wild type. (10)

Drosophila served as an excellent model for investigating effects with various genes, with 77% of human disease genes matched in *Drosophila* genome (9), which makes *Drosophila* a very powerful research organism. Also, due to their short generation time and ease of care, rearing large population of *Drosophila* is much easier than any other mammalian organisms. Although mammalian lipid biosynthesis is very complex and the fate of products are different, the pathways where unsaturated fatty acid catalyze into monounsaturated fatty acid is highly similar between mammals and *Drosophila*.

In this study we used ribonucleic acid interference (RNAi) stock line from Vienna *Drosophila* RNAi Center (VDRC). They used a GAL4/UAS (Upstream activating sequences) binary expression method to achieve inactivation using fragments of short clones as inverted repeats. The GAL4/UAS RNAi system is a method commonly used in *Drosophila.* To activate the interference, two different stocks are used. One is the GAL4 driver which had a tissue specific promoter, the other stock contains inverted repeats under the control of UAS yeast premotor with binding sites for GAL4. When the GAL4 driver is crossed with the UAS stock, the offspring of these crosses will have both pieces of the puzzle and the targeted gene will be silenced in tissues where GAL4 is expressed. (3)

The goal of this study is to address the influences of delta-9 desaturases on offspring productions. Some of our key results are coherent with previous studies. Shorter lifespan has been observed with KD groups of both Desat, which is compatible with *C. elegans* studies that also showed animals with mutations that hinder delta-9 desaturases expression. However, impacts of delta-9 desaturases on fecundity have not been observed until now.

Methods

I. Fly populations

1. Maintenance and rearing experimental lines.

Two different diets were used. One is the yellow cornmeal diet called regular food, which contains yellow cornmeal, tortula yeast, molasses, agar, propionic acid and tegosept. The other is a defined diet called minimum diet, which contains glucose, Bacto yeast extract, agar, propionic acid and tegosept. (See appendix I and II for complete recipe.)

All RNAi lines were ordered from VDRC stock center. After quarantine to ensure no parasites were present, flies were reared on regular food at 25°C unless otherwise noted. To collect virgins for parental generation, twenty five male and twenty five female stock line flies were placed on glass cut bottles for an egg laying period of forty eight hours. After egg laying, flies were transferred to new bottles if more eggs were needed. Eggs were then collected under microscope. One hundred eggs were placed into new food vials, with thirty vials for each line. In about ten to twelve days the F1 generation emerged. When pupae turned into a darker brown or black color, vials were checked and placed in a 17°C incubator. Vials were checked again every eight hours, and all emerged flies were

sorted to separate male from female. Each sorted vial contained fifty virgin flies: males or females respectively.

After the desired number of virgin flies was collected, sorted vials with females were watched for five days to ensure they are virgins. Then crossed according to table 1 for knocked down F2. Control group was generated by crossing Control W1118 flies with driver Tub GaL 4 flies, this was to ensure control group contains the same genetic back ground as experimental group. RNAi lines were generated by crossing either Desat 1 (CG5925) or Desat 2 (CG5887) flies with Tub GaL 4 flies. Egg collection and rearing were the same as F1 generation.

Table 1 Crosses for Control and Knocked down (KD) groups.

Lines	Control	Desat 1 KD	Desat 2 KD
Crosses	CMXDF DMXCF	CG5925 M X DF DM X CG5925 F	CG5887 M X DF DM X CG 5887 F

Control Males (CM) are crossed (X) with Driver Females (DF), Driver Males (DM) are crossed with Control Females (CF) for complete genetic background of knocked down groups, progeny from these crosses will served as control groups for all studies. For Desat 1 KD are progeny from crosses of CG5925 males crossed with DF and CG5925 females crossed with DM. CG5887 males crossed with DF and CG5887 crossed with DM produced progeny for Desat 2 KD.

2. Fly collection for lipid composition and qPCR.

qPCR flies were collected at day five of fly emergence, flies were flash frozen using liquid nitrogen and decapitated by shaking frozen flies in a vial. Once the head was removed, ten flies were collected for each sample. Flies collected for lipid profile analysis (lipid flies) were collected using the method above on day five and day thirty. Lipid flies were kept in longevity cages after day seven and maintained in the same manner as longevity study. Each line contained three replicates.

Larvae hatched from eggs produced via crosses mentioned above in Table 1 were placed onto minimum low fat diet and checked development process every twelve hours. Larvae between second instar and third instar were collected at time where fifty percent death of larvae occurred. These larvae were place in 2ml tubes in groups of ten and flash freeze with liquid nitrogen for lipid extraction.

II. qPCR.

RNA extractions were conducted following Qiagen RNeasy kit, and cDNA were produced using Bio-Rad iScript kit to generate a template. RT-PCR were performed on 96-well plate using Eppendorf cycle. Each reaction contained: 2µl RNA free water, 5µl syber green, 1µl forward primer at 2.5µm, 1µl reverse primer at 2.5µm and 1µl cDNA template. Ribosomal protein 49 was chosen as the standard marker.

III. Fecundity and longevity study.

1. Fecundity study.

Within twelve hours of F2 fly emergence, males and females were separated by lightly anesthetized with diethyl ether, sexing and placing about twenty five flies per vial. Flies were then watched closely to ensure virginity and let mature until day five after emerging.

Single pair mating was set up with one female fly and one male fly according to Table 2. After twenty four hours, the male fly was removed and discarded. The female fly was placed onto new food, and transferred onto new food every twenty four hours for fourteen days. Eggs from each old vial were counted and recorded until day fourteen. Progeny was then counted and recorded on day fifteen after the eggs were laid to ensure no more pupae emerged. Each line contained ten samples per sex.

Table 2 Crosses for fecundity study.

Male	Cross with
Desat 1(M)X Driver(F)	CNS(F)
Driver (M)X Desat 1(F)	CNS(F)
Desat 2 (M)X Driver(F)	CNS(F)
Driver (M)X Desat 2(F)	CNS(F)
Control W1118 (M)X Driver(F)	CNS(F)
Driver (M)X Control W1118(F)	CNS(F)

Cross with	Female
CNS(M)	Desat 1(M)X Driver(F)
CNS(M)	Driver (M)X Desat 1(F)
CNS(M)	Desat 2 (M)X Driver(F)
CNS(M)	Driver (M)X Desat 2(F)
CNS(M)	Control W1118(M)X Driver(F)
CNS(M)	Driver (M)X Control W1118(F)

In male fecundity study, male progeny from Desat 1 (CG5925) males (M) crossed with Driver females (F); or Driver females (F) crossed with Desat 1 females (F) are crossed with CNS female (F). Male progeny of Desat 2 (CG5887) males crossed with Driver females (F) or Driver males (M) crossed with Desat 2 females (F) are crossed with CNS females (F). For control groups, male progeny of Control W1118 males (M) crossed with Driver females (F) or Driver males (M) crossed with Control W1118 females (F) are crossed with CNS female (F). Female fecundity follows the same procedure except all female progeny from various crosses are crossed with CNS male (M).

2. Longevity study

On day three of F2 fly emergence, male and female flies were separated by anesthetized with diethyl ether and separated with fifty male or female flies per vial. Sorted flies were given forty eight hours to recover, the set up in individual fly cages. (See Figure 1) Each cages contain fifty flies, male or female only, each sex had three cages per line. (See Table 3) Every forty eight hours the number of dead flies was counted and removed, fresh food media was exchanged for the old food vials until all flies in the cage were dead.

IV. Lipid analysis

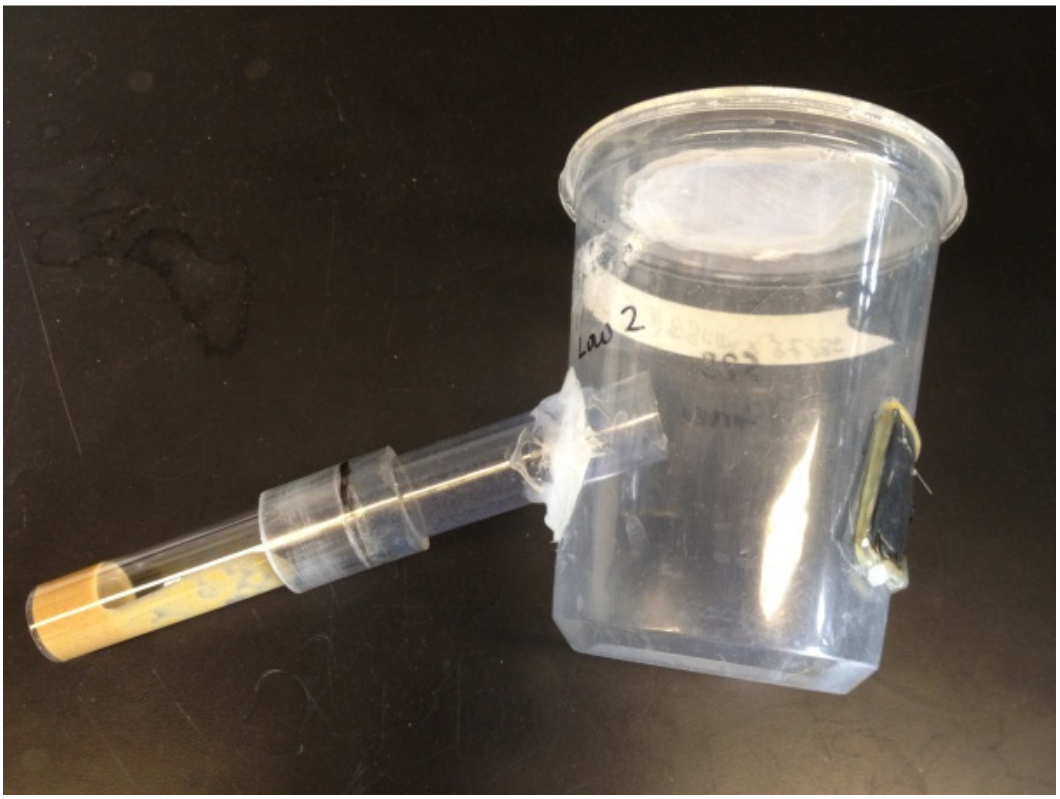
Lipid extractions were conducted following Bligh and Dyer, using ten decapitated flies. Then GC fatty acid methyl ester analysis-acidified methanol was used as described in appendix II and III. Final product was suspended in 0.1ml of methyl acetate and run on GC-Mass spectrometer. Lipid composition used ten flies for each sample; each line/sex had three samples.

Lipid analysis followed procedures in appendix II and III used minimum fat diet and regular medium, each sample contains 1 ml of medium with three samples each.

V. Survival on minimum diet

Twenty five fly eggs were place on minimum diet as described. Vials were checked every twenty four hours for larvae activity and the final number of adult emerged was recorded. Each line contained five samples.

Figure 1 Fly cage.



Fly cages are made with plastic cups with one panel window consisted of rubber square with a “X” shape opening to prevent flies from escaping and easy removal of flies with insertion of a aspirator. There is another opening with an extended tube attached with hot glue for food media access. The cover of cages are cut and cover with cheese cloth to ensure air circulations.

Table 3 Longevity crosses.

Male/ Female
Desat 1(M)X Driver(F)
Driver (M)X Desat 1(F)
Desat 2 (M)X Driver(F)
Driver (M)X Desat 2(F)
Control W1118 (M)X Driver(F)
Driver (M)X Control W1118(F)

Longevity study of Desat 1 KD are conducted with fifty male or female progeny from crosses of Desat 1 (CG5925) males (M) crossed with Driver females (F) or Driver males (M) crossed with Desat 1 females (F). Desat 2 KD consisted fifty progeny of male or female from crosses with Desat 2 (CG5887) males (M) crossed with Driver females (F) or Driver males (M) crossed with Desat 2 females (F). Control group contained fifty male or female progeny from crosses of Control W1118 males (M) crossed with Driver females (F) or Driver males (M) crossed with Control W1118 females (F).

Results

I. Rearing experimental flies.

When experimental flies were collected, 30.9% of Desat 1 KD flies failed to eclosed, and 24.6% of Desat 2 KD flies did not eclosed as well. While only 4.7% of Control flies did not eclosed. (Fig. 2)

II. qPCR.

In order to ensure experimental flies are knocked-down with respect to the RNA suppression, qPCR was performed and the expression level of two desaturases were compared to control group. The qPCR results showed that the strains are both knocked-down for Desat 1 and Desat 2. In Desat 1 KD male, expression level of Desat 1 compared to Control group is 16.9% and in Desat 2 male KD is 7.54%. (Fig. 3) As for Desat 1 female KD, expression level compared to Control group is 24.3% and Desat 2 KD female is 9.9%. (Fig. 4)

III. Fecundity and longevity study.

To examine the influences on disrupted desaturases on life history traits of *Drosophila*, fecundity and longevity studies were performed. In fecundity trials, egg production by knocked-down females decrease significantly from day 2 throughout day 14, with exceptions of Desat 2 female KD in day 12. (Fig. 5) In progeny counts, all days except day 12 showed significant decrease of emergence rate for knocked-down females. (Fig. 6)

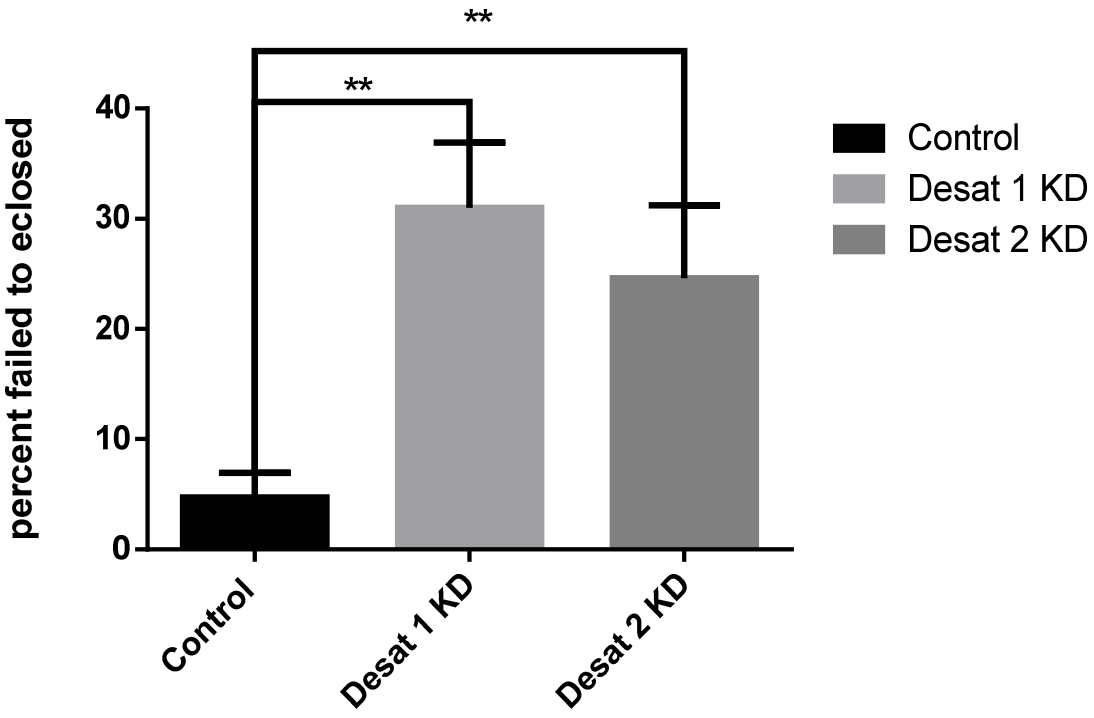
The longevity experiments showed significant die off starting on day 45 and the knocked-down group in both male and female populations were dead by day 50 and day 55. The control groups in male and females lived to about 100 days. (Fig. 7 & 8)

IV. Lipid analysis.

To further understand the role dela-9 desaturases play in *Drosophila* lipid metabolism, a series of lipid analysis were conducted. In C16, there is a significant decrease of five days old Desat 1 KD in males when compared to control group. There is a 4% decrease in Desat 1 males KD in day five. (Fig. 9) Although there is no significance for Desat 1 and Desat 2 female KD in both day five and day thirty. (Fig. 10) For C16:1, there is a 7% decrease in Desat 1 KD males and 9% for Desat 2 KD males. A 6.5% decrease in Desat 1 KD males and 6% decrease of thirty days old Desat 2 KD males (Fig. 11) and in females there are significant decreases in both five day old and thirty days old flies. (Fig. 12) The decreases for five days old and thirty days old for C16:1 are 3% and 5% for Desat 1 KD females and 5% and 4% for Desat 2 KD females. Markable increases of C18 is observed in thirty days old male flies, 13% for Desat 1 KD and 8% for Desat 2; Desat 2 KD had a 3.5% decrease in day five. (Fig. 13) As for females, buildups of C18 are observed in both five days old and thirty days old flies; 2% and 7% for Desat 1 KD and 8% and 7% for Desat 2KD. (Fig. 14) In C18:1 Desat 2 KD male had an increase at five days old of 5% and Desat 1 KD males had a slight increase of 1.6% at day five and a decrease of 4% at thirty days old. (Fig. 15) In females there are a general increase at five days old and decreases at thirty days old in both Desat 1 and Desat 2 KD. (Fig. 16) There are 5% and 6% increase of C18:2 for Desat 1 and Desat 2 KD males at five days old, and 2% decrease of Desat 1 KD at thirty days old. (Fig. 17) In five days old Desat 2 KD, there is a 4.5% decrease and 3% decrease in thirty days old KD females. (Fig. 18)

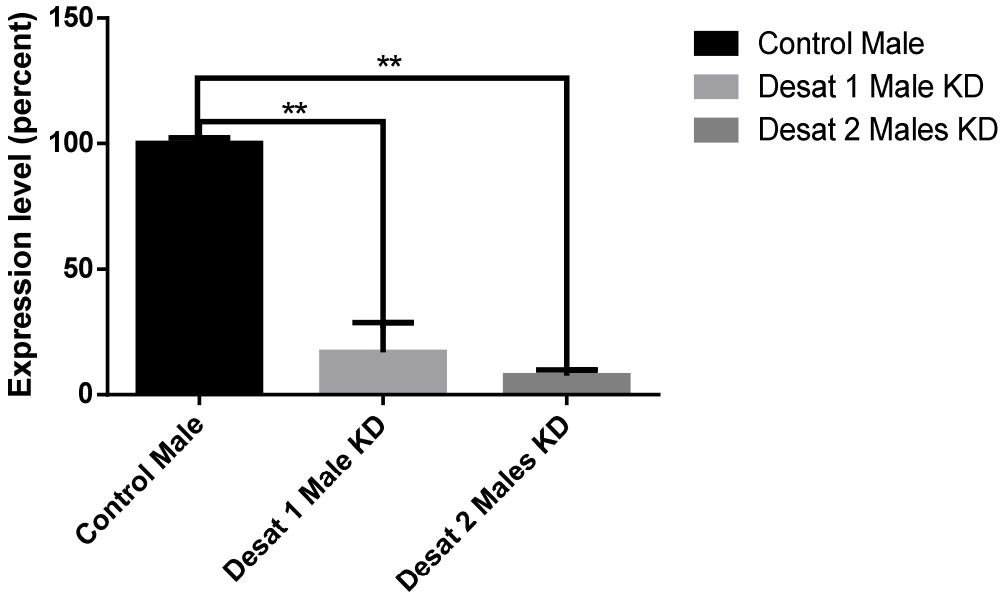
A set of lipid profile analysis were also performed to investigate the difference in lipid contents of two diets. Differences in lipid concentration are more than tenfold for C16, C18 and C18:1. The concentrations of C14, C14:1, C16:1 and C18:2 are non-existent in minimum low fat diet compared with the regular diet. (Fig. 19)

Figure 2 Percent of flies failed to eclosed.



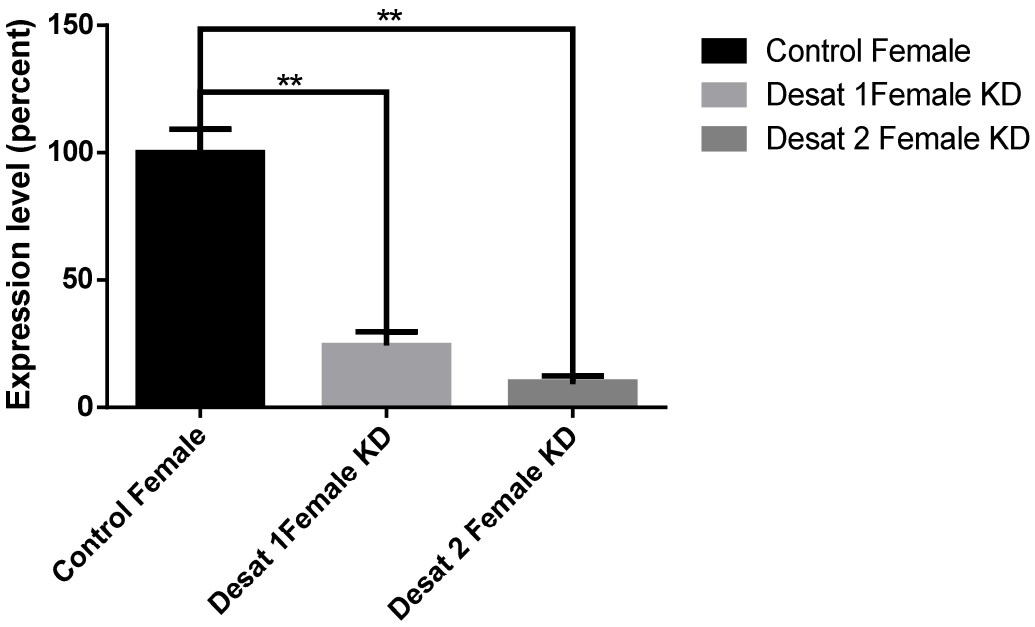
When rearing experimental flies on regular diet, portions of fly population failed to eclose. In control group, only 4.7% did not eclose, while in Desat 1 KD 30.9% and Desat 2 KD 24.6% failed to eclose. T-tests were performed to compare Control and Desat 1 KD or Desat 2 KD. Results showed p-values are less than 0.001 in both. ** denoted p-value less than 0.001.

Figure 3 Male qPCR for Control, Desat 1 KD and Desat 2 KD.



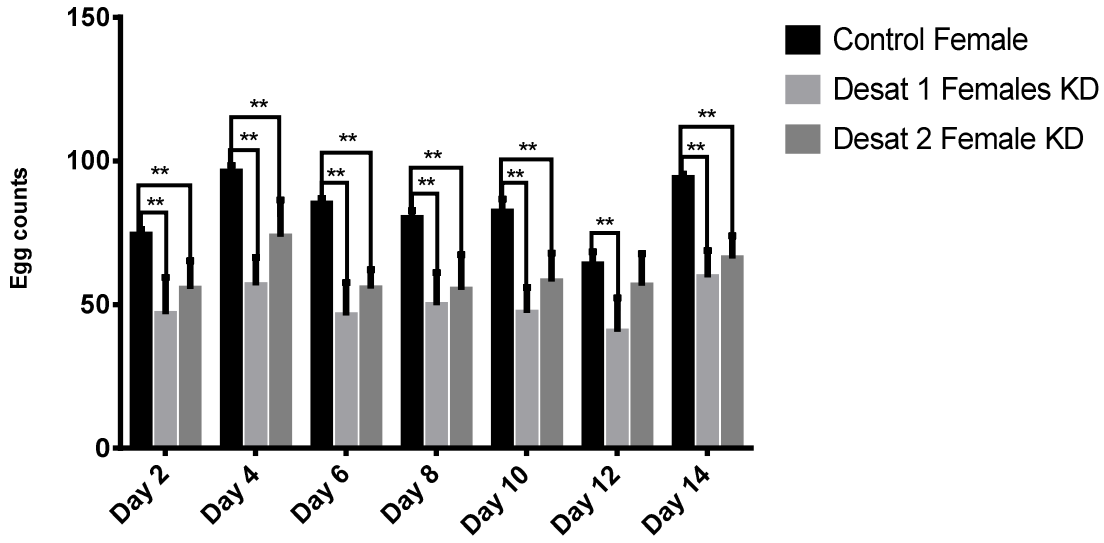
When expression level of control males are set to be 100%, the expression level for Desat 1 male KD is 16.9%, and Desat 2 male KD is 7.54%. T-test was performed to comparisons Desat 1 male KD with Control male and Desat 2 male KD with Control male, both results showed p-value is less than 0.001. ** denotes p-value less than 0.001.

Figure 4 Female qPCR for Control, Desat 1 KD and Desat 2 KD.



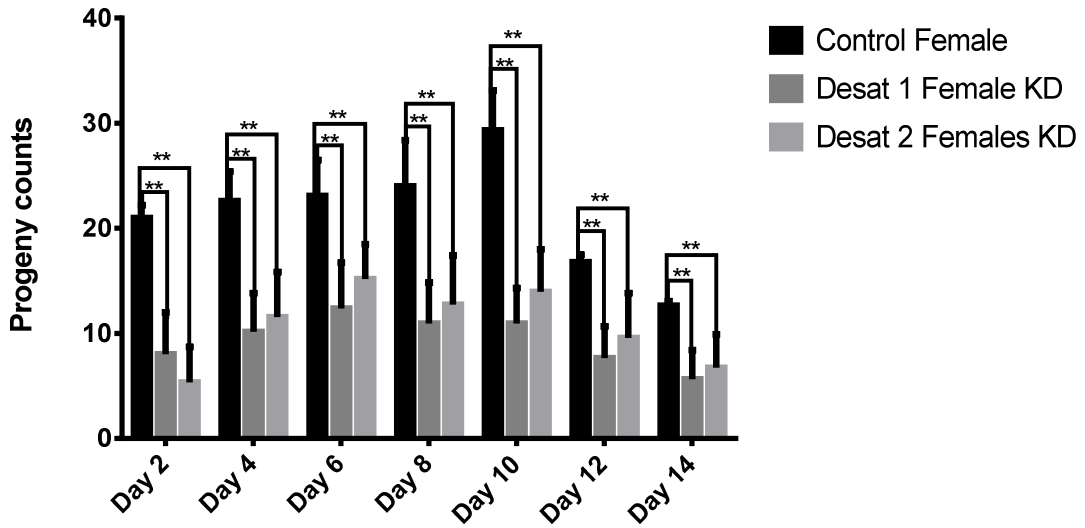
When Control female expression level is set for 100%, Desat 1 female KD expression level is 24.3%, and Desat 2 female KD is 9.9%. T-test was performed to comparisons Desat 1 female KD with Control female and Desat 2 female KD with Control female, both results showed p-value is less than 0.001. ** denotes p-value less than 0.001.

Figure 5 Egg counts for Control Females, Desat 1 Females KD and Desat 2 Females KD.



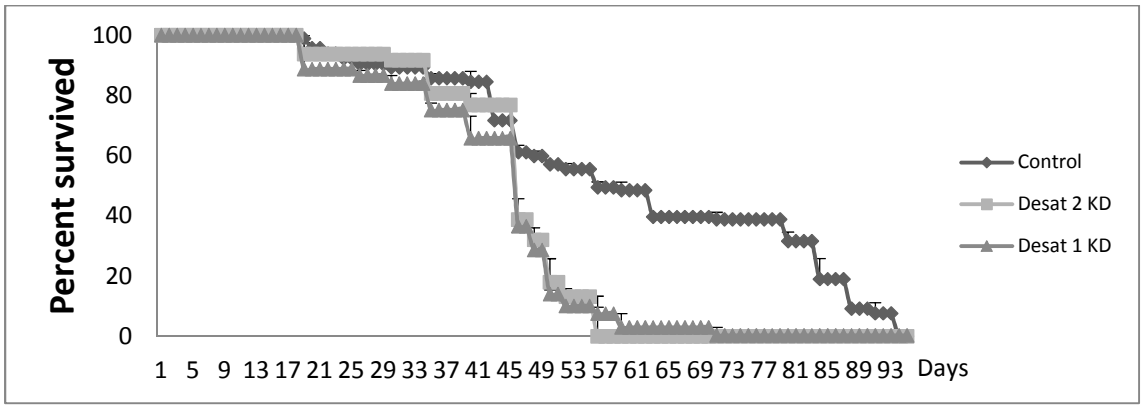
Fecundity study were conducted with crosses mentioned in methods. Significant decreases in egg productions were observed throughout 14 days with exception of Desat 2 female KD in day 12. T-test was used for comparisons of control group with Desat 1 Females KD or Desat 2 Females KD individuality for each data point. ** denoted p-value less than 0.001.

Figure 6 Progeny count for Control Female, Desat 1 Females KD and Desat 2 Females KD.



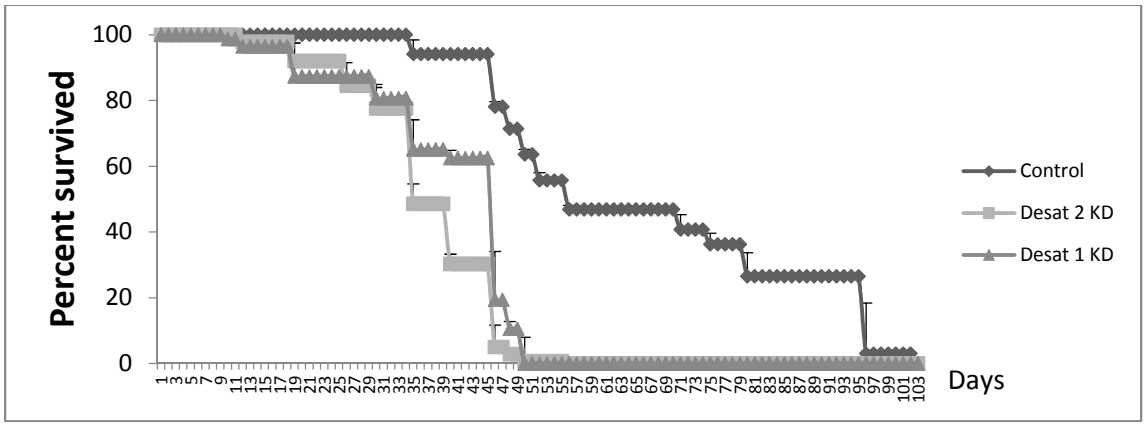
Progeny counts were conducted as mentioned in methods, significant decreases of progeny eclosed were observed from Day 2 until Day 14. T-test was used for comparisons of control group with Desat 1 Females KD or Desat 2 Females KD individuality for each data point. ** denoted p-value less than 0.001.

Figure 7 Longevity study for Control Males, Desat 1 Males KD and Desat 2 Males KD.



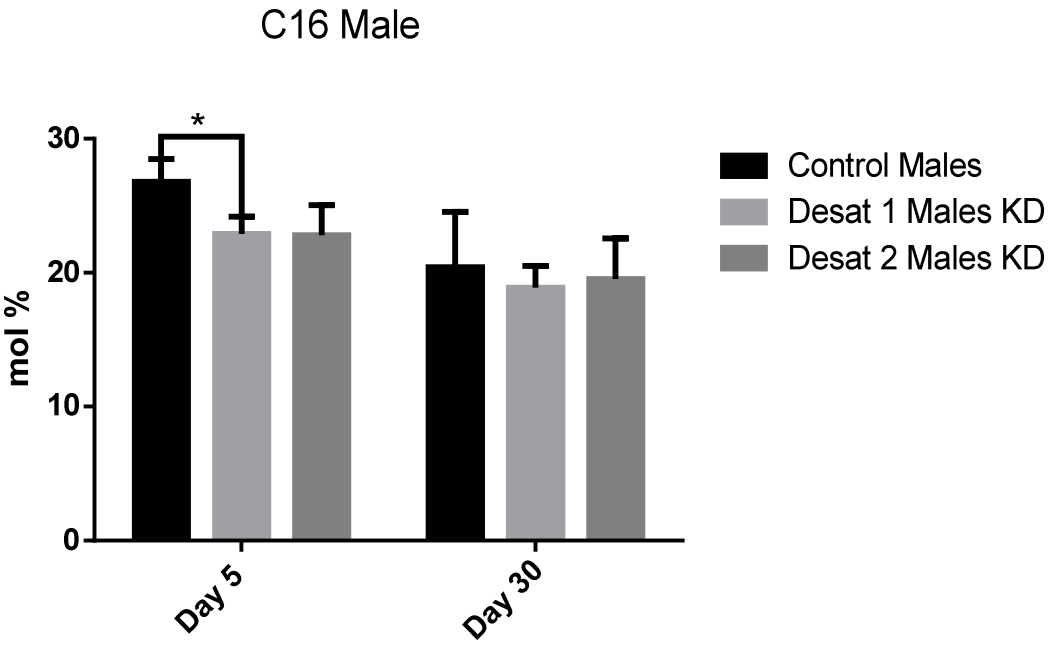
Mortality rates between control and the two knock down lines are significantly different, the two knock down lines are having much shorter life span. This is true in both male and female. Each data point has 3 samples and each sample population started with 50 flies. Food media was replaced every 48 hours until all the flies died.

Figure 8 Longevity study for Control Females, Desat 1 Females KD and Desat 2 Females KD.



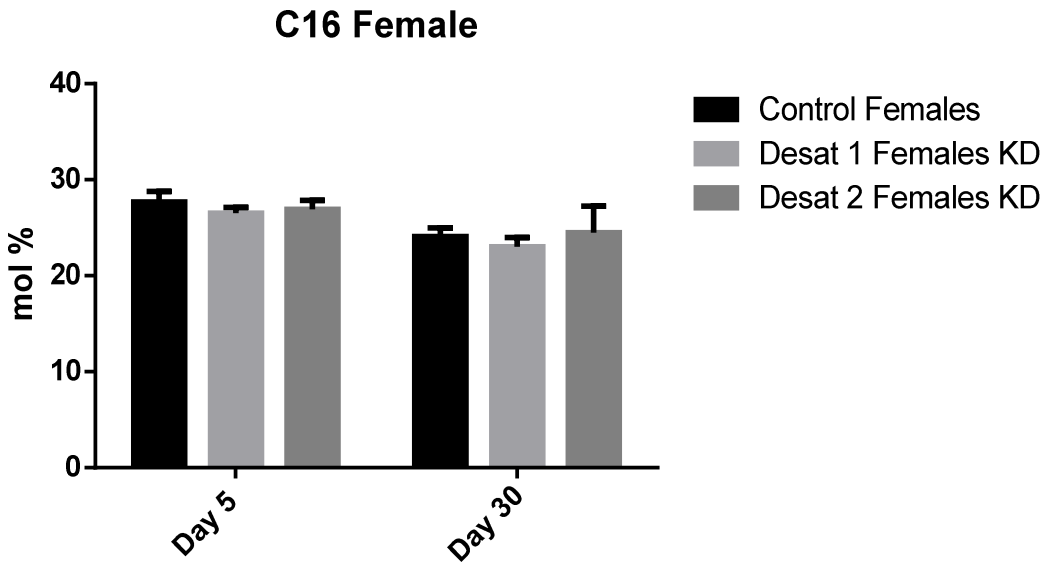
Mortality rates between control and the two knock down lines are significant different, the two knock down lines are having much shorter life span. This is true in both male and female. Each data point has 3 samples and each sample population started with 50 flies. Food media was replaced every 48 hours until all the flies died.

Figure 9 C16 Lipid composition of Control Males, Desat 1 Males KD and Desat 2 Males KD.



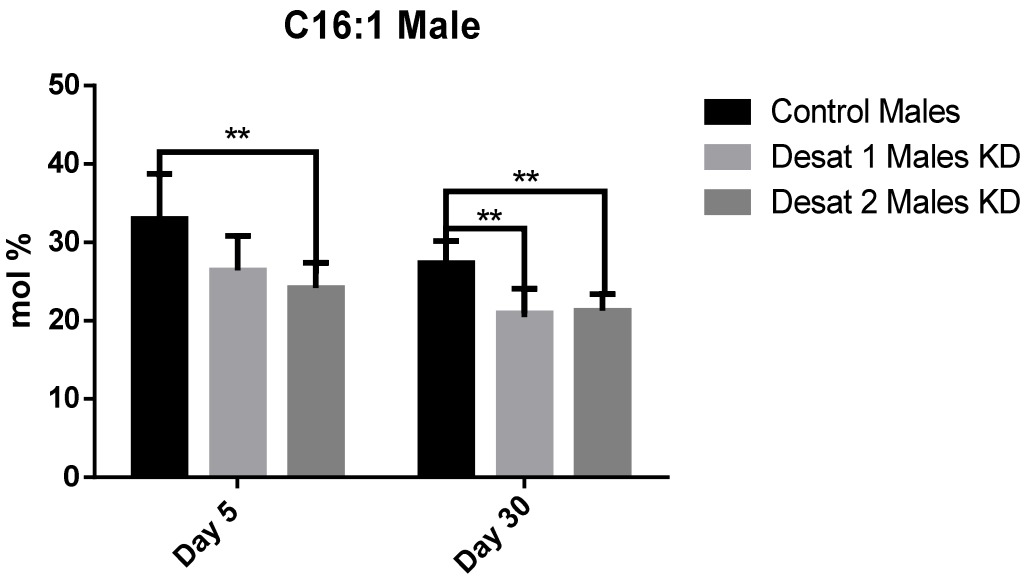
When compare Control males lipid composition in terms of mole percent, 4% decrease in Desat 1 Males KD of C16 in day 5 was observed. T-tests were perform to compare Control Males with either Desat 1 KD or Desat 2 KD at each data point. * denoted p-value less than 0.05.

Figure 10 C16 Lipid composition of Control Females, Desat 1 Females KD and Desat 2 Females KD.



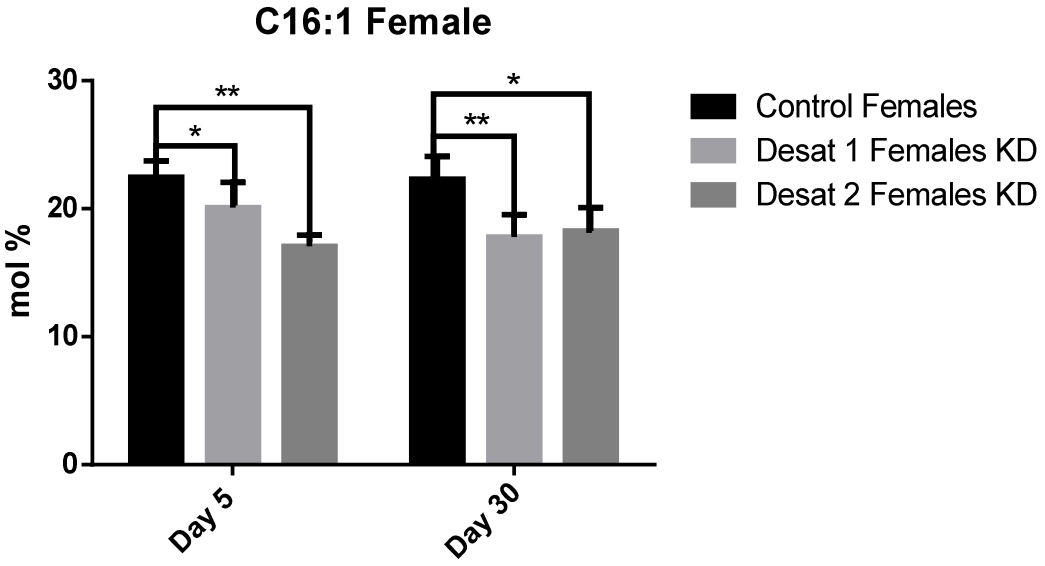
When compare Control females lipid composition in terms of mole percent, slight decrease in Desat 1 Females KD of C16 in day 5 and day 30 were observed. Small decrease in Desat 2 Females KD was shown and some increases in Day 30. T-tests were perform to compare Control Females with either Desat 1 KD or Desat 2 KD at each data point.

Figure 11 C16:1 Lipid composition of Control Males, Desat 1 Males KD and Desat 2 Males KD.



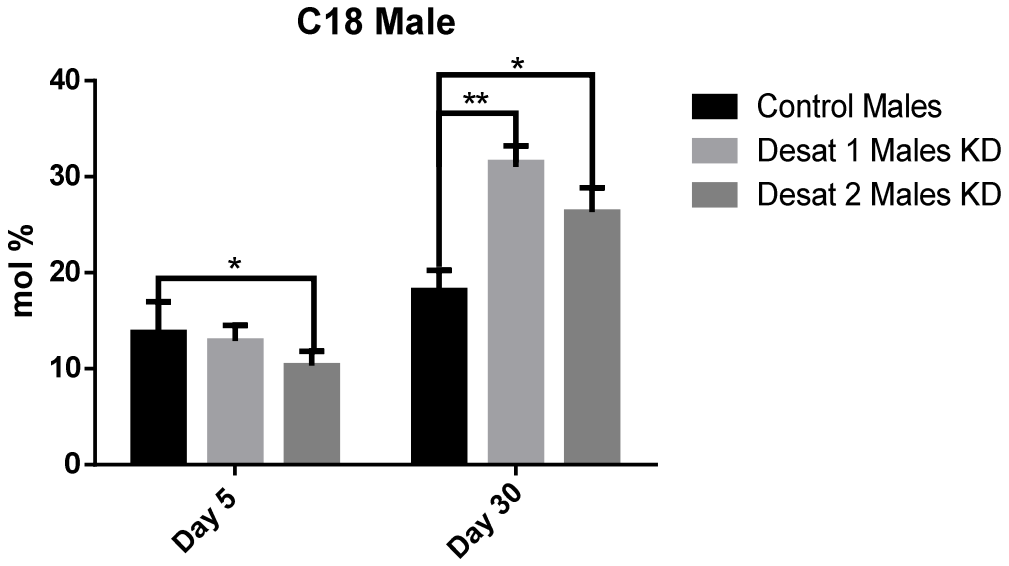
When compared to Control group, there is a 7% decrease in Desat 1 KD males and 9% for Desat 2 KD males. A 6.5% decrease in Desat 1 KD males and 6% decrease of thirty days old Desat 2 KD males. T-tests were performed to compare Control Males with Desat 1 Males KD or Desat 2 Males KD at each data points. In Desat 1 Males KD at day five, p-value is very close to 0.05. At all other data points p-values are less than 0.01. ** denoted p-value less than 0.01.

Figure 12 C16:1 Lipid composition of Control Females, Desat 1 Females KD and Desat 2 Females KD.



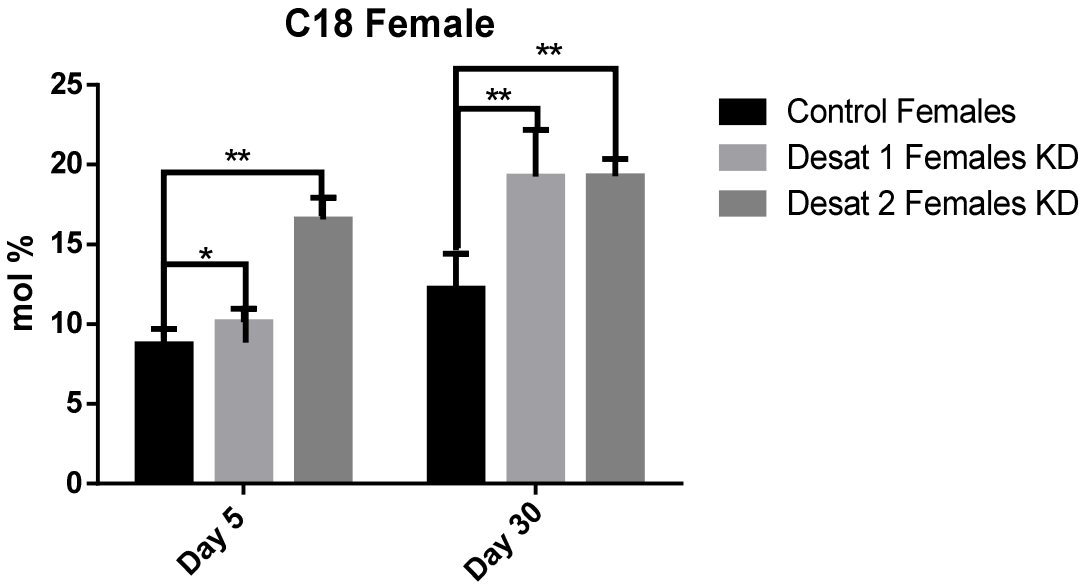
When compared to Control Females, the decreases for five days old and thirty days old for C16:1 are 3% and 5% for Desat 1 KD females and 5% and 4% for Desat 2 KD females. T-tests were perform to compare Control Females with either Desat 1 KD or Desat 2 KD at each data point. ** denoted p-value less than 0.01 and * denoted for p-value less than 0.05.

Figure 13 C18 Lipid composition of Control Males, Desat 1 Males KD and Desat 2 Males KD.



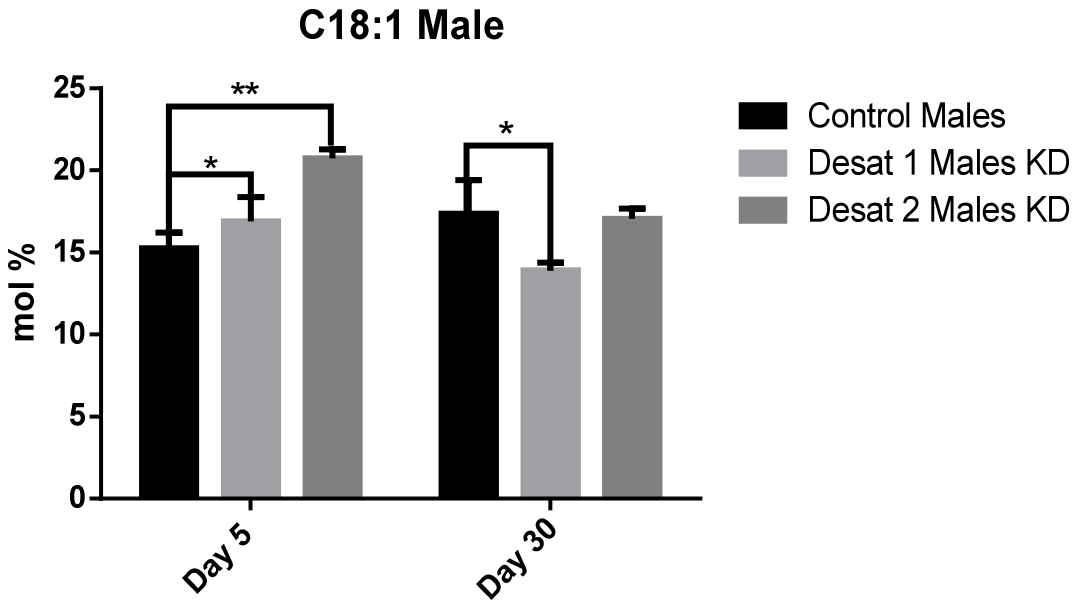
When compared to Control Males, there is a 3.5% decrease for Desat 2 KD in day five. Markable increases of C18 is observed in thirty days old male flies, 13% for Desat 1 KD and 8% for Desat 2. T-tests were performed to compare Control Males with Desat 1 Males KD or Desat 2 Males KD at each data points. ** denoted p-value less than 0.01 and * denoted for p-value less than 0.05.

Figure 14 C18 Lipid composition of Control Females, Desat 1 Females KD and Desat 2 Females KD.



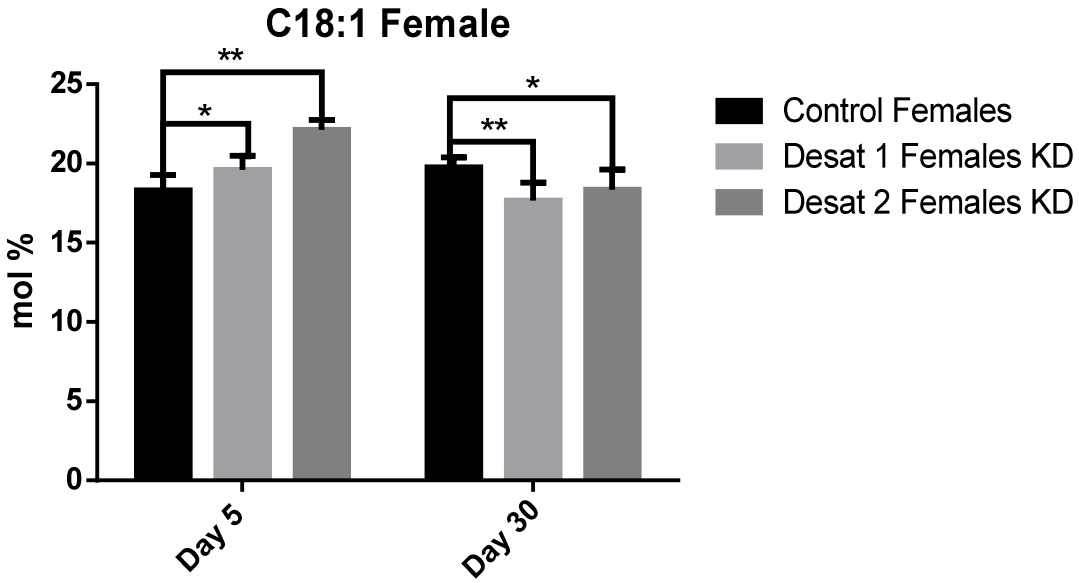
When compared to Control Females, buildups of C18 are observed in both five days old and thirty days old flies; 2% and 7% for Desat 1 KD and 8% and 7% for Desat 2KD. T-tests were perform to compare Control Females with either Desat 1 KD or Desat 2 KD at each data point. ** denoted p-value less than 0.01 and * denoted for p-value less than 0.05.

Figure 15 C18:1 Lipid composition of Control Males, Desat 1 Males KD and Desat 2 Males KD.



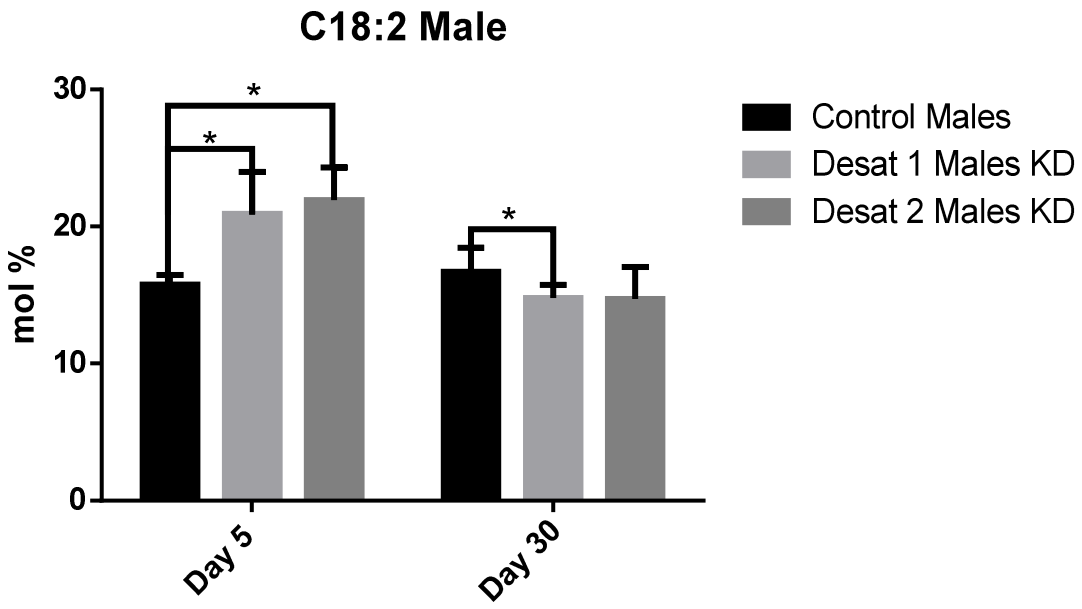
In C18:1 Desat 2 KD male had an increase at five days old of 5% and Desat 1 KD males had a slight increase of 1.6% at day five and a decrease of 4% at thirty days old. T-tests were performed to compare Control Males with Desat 1 Males KD or Desat 2 Males KD at each data points. ** denoted p-value less than 0.01 and * denoted for p-value less than 0.05.

Figure 16 C18:1 Lipid composition of Control Females, Desat 1 Females KD and Desat 2 Females KD.



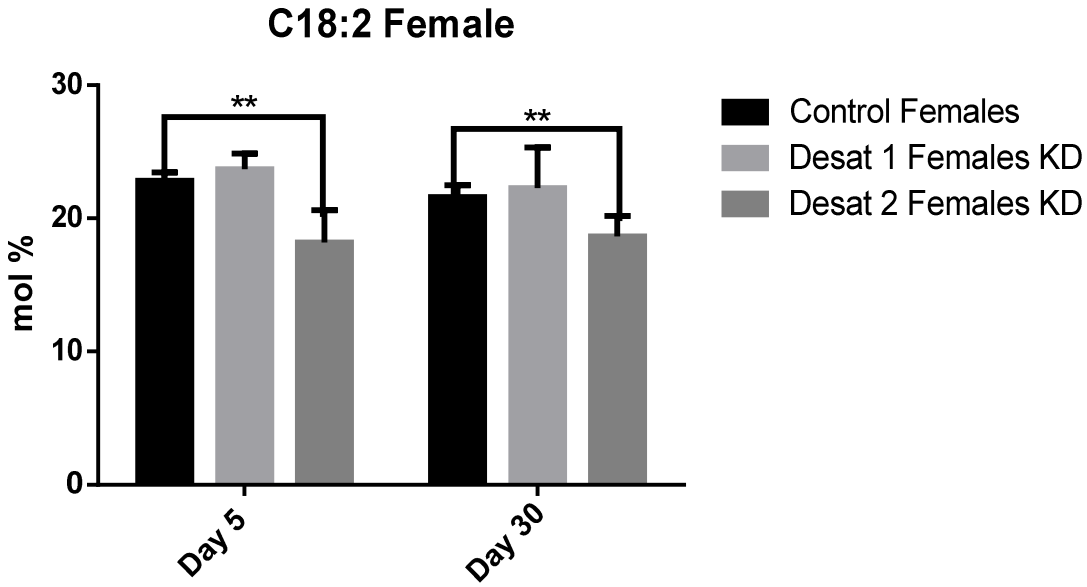
When compare to Control Females, there are a general increase at five days old and decreases at thirty days old in both Desat 1 and Desat 2 KD. Desat 1 Females KD had a 1.3% increase and 2% decrease at day five and day thirty. In Desat 2 Females KD, there is an increase of 3.8% increase and a decrease of 1.4% in day five and day thirty. T-tests were perform to compare Control Females with either Desat 1 KD or Desat 2 KD at each data point. ** denoted p-value less than 0.01 and * denoted for p-value less than 0.05.

Figure 17 C18:2 Lipid composition of Control Males, Desat 1 Males KD and Desat 2 Males KD.



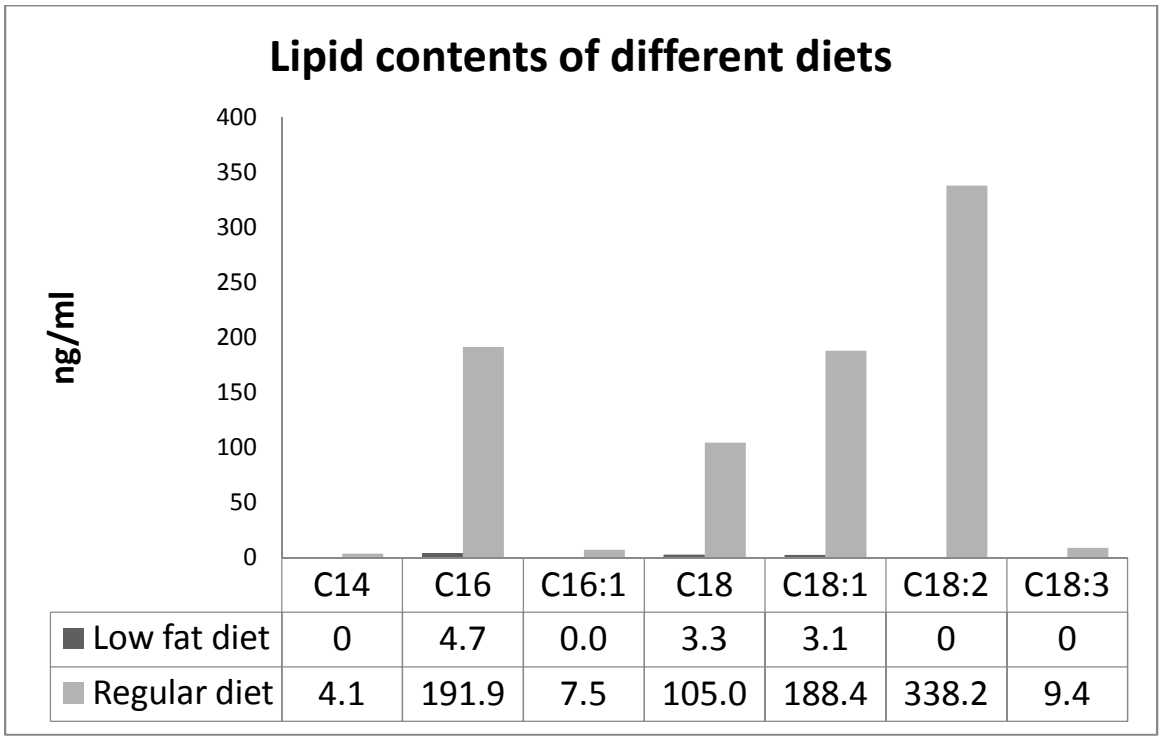
When compared to Control Males, there are 5% and 6% increases of C18:2 for Desat 1 and Desat 2 KD males at five days old, and 2% decrease of Desat 1 KD at thirty days old. T-tests were performed to compare Control Males with Desat 1 Males KD or Desat 2 Males KD at each data points. * denoted for p-value less than 0.05.

Figure 18 C18:2 Lipid composition of Control Females, Desat 1 Females KD and Desat 2 Females KD.



When compare to Control Females, five days old Desat 2 KD, there is a 4.5% decrease and 3% decrease in thirty days old KD females. T-tests were perform to compare Control Females with either Desat 1 KD or Desat 2 KD at each data point. ** denoted p-value less than 0.01.

Figure 19. Lipid composition of regular and low fat (minimum) diet.



	C14	C16	C16:1	C18	C18:1	C18:2	C18:3
Low fat diet	0	4.7	0.0	3.3	3.1	0	0
Regular diet	4.1	191.9	7.5	105.0	188.4	338.2	9.4

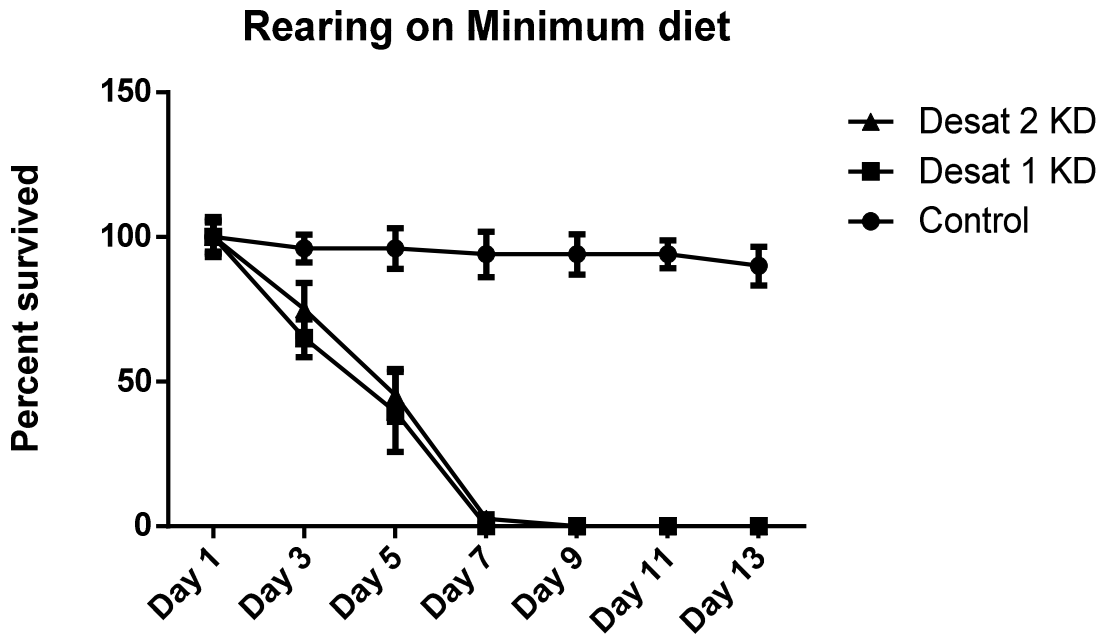
Lipid extractions were conducted as described with 1 ml of food media. Regular diet contains large amount of lipid in comparison to minimum low fat diet. For C16, C18 and C18:1, the difference between diets are more than 10 fold.

V. Rearing on minimum diet.

When raised on minimum diet, both Desat 1 and Desat 2 knocked-down has zero eclosion rate and died out at day seven. Control group showed about 90% emerged rate into adult flies. (Fig.20) When 750mmol oleic acid was added to minimum diet, 16% of Desat 1 KD and 23% of Desat 2 KD developed into pupae. (Fig. 21)

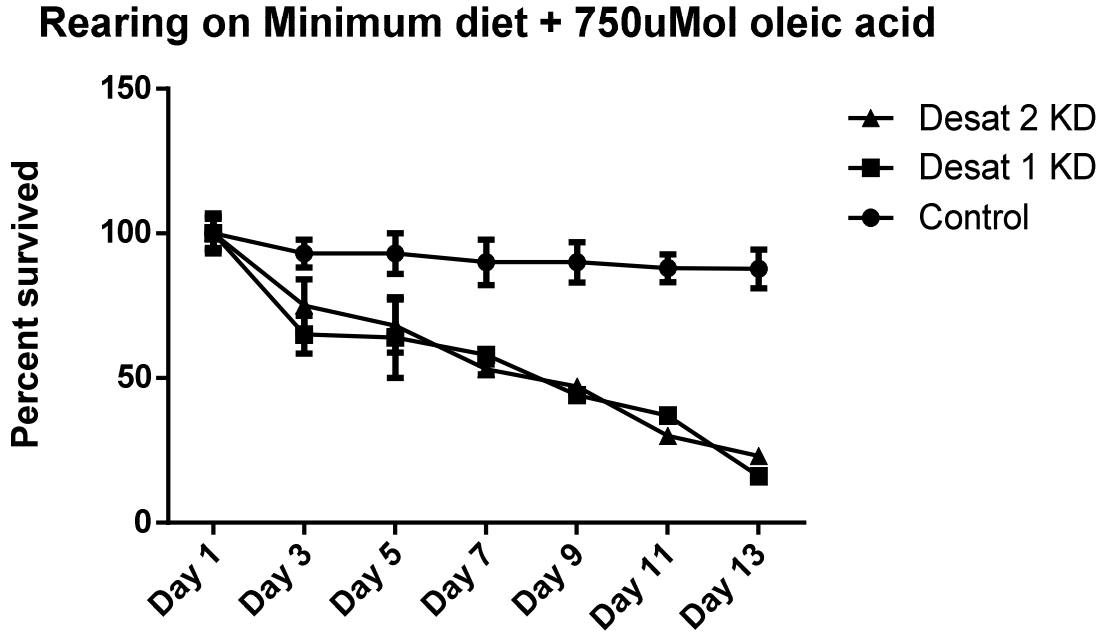
To further investigate reason behind this phenomenon of development difficulty, larvae were collected at time point where 50% death occurred and lipid composition studies were run using GC. We observed markable changes in lipid composition from these larvae. (Fig. 22 & 23)

Figure 20 Larvae survival rate when rear on minimum diet.



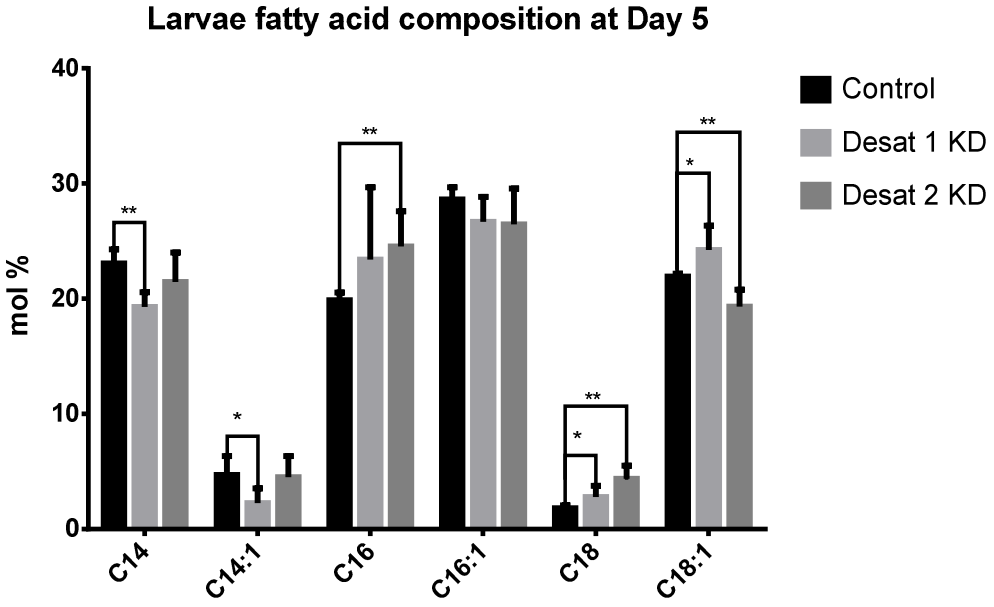
When eggs are placed on to minimum low fat diet, development of larvae slowed down since there is not enough nutrients. However, Control group still pupae at day 13, where both Desat 1 KD and Desat 2 KD starting to died off at day 5 and died out on day 7.

Figure 21 Larvae survival rate when rear on minimum diet with 750 mmol oleic acid.



When eggs are placed onto minimum low fat diet with 750 mmol of oleic acid additive. Control group pupation rate was 87% and the Desat 1 KD and Desat 2 KD pupae at 16% and 23%.

Figure 22. Lipid composition of five day old larvae. (mol %)

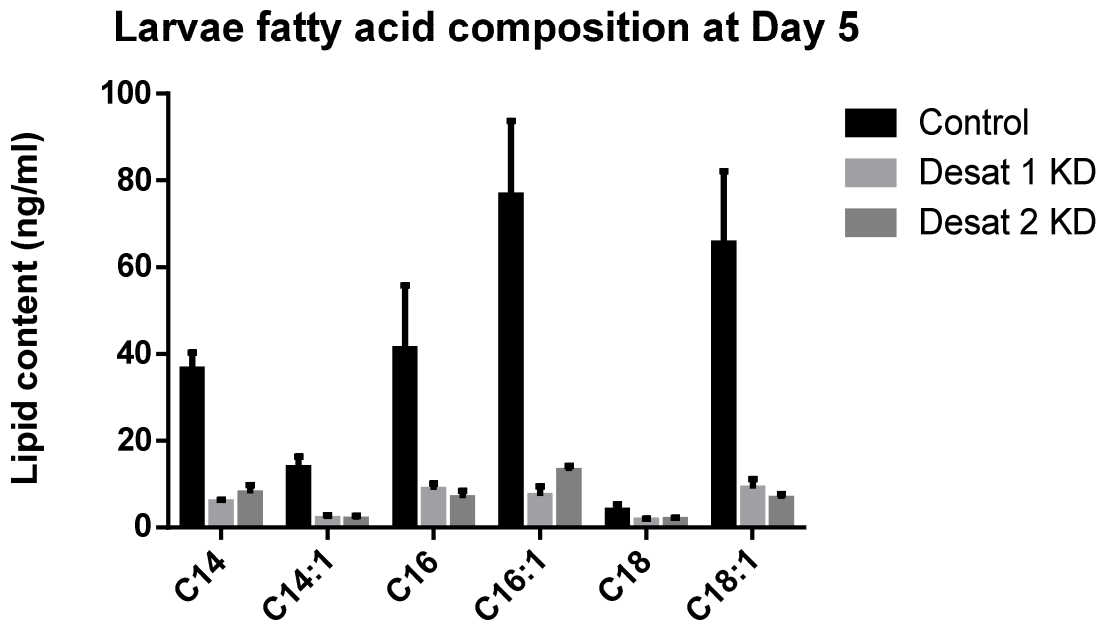


When compared to control group, lipid composition of Desat 1 KD and Desat 2 KD larvae had various changes. However, the KD flies overall maintained a similar composition compared to control group.

Both KD showed decreases in C14 and C16:1, some build ups in C16 and C18. Significant decreases are seen in Desat 1 KD on C14, C14:1, increases in C18 and C18:1. For Desat 2 KD, significant increases were observed in C16 and C18, decreases in C18:1.

T-tests were performed for comparison between Control group and Desat 1 KD or Desat 2 KD in every fatty acid category individually. ** denoted p-value less than 0.01 and * denoted for p-value less than 0.05.

Figure 22. Lipid composition of five days old larvae. (Ng/ml)



When compared to control group, lipid composition of Desat 1 KD and Desat 2 KD larvae had markable lower concentrations. T-tests were performed for comparison between Control group and Desat 1 KD or Desat 2 KD in every fatty acid category individually. All p-values were less than 0.01 in this figure.

Discussion

I. qPCR and Fly population.

qPCR results showed that the experimental flies are truly knocked-down. Difficulty in eclosion has been observed when rearing knocked-down line on regular diet. Approximately 25-30 percent of the knocked-down F2 generation failed to eclose. Therefore, all studies were conducted in a sub-population of F2 generation. Among the flies that did not eclose successfully, some of them did not eclose at all and the others seem to have gotten stuck during eclosion. This can result from lack of lubrication or structural change in pupae shells, since disrupted delta-9 desaturases may interfere with structural changes from cuticle to pupal cases. Delta-9 desaturases may play an important role in long chain hydrocarbons. Since *Drosophila* may follow the similar pathway in mammals to decarboxylate MUFA into monoenes. (2) Previous studies have shown that 20-Hydroxyecdysone is required for pupal through adult molting in *Drosophila*. (4) Disruption of delta-9 desaturase will interfere with cholesterol production from unsaturated fatty acids. (8)

II. Fecundity and longevity study.

Observations from fecundity studies further support the importance of delta-9 desaturases in *Drosophila*. Limitations of unsaturated fatty acid conversion hinders egg production and viability. Studies in mice have shown that without delta-9 desaturase animals will not be able to synthesize TAG. (7) TAG being the main energy storage in many organisms suggests that the impact on lifespan, especially in female KD groups, may result from investing energy into offspring production. Decreased number of progeny can also be impacted by disruptions of delta-9

desaturaes, since these enzymes contribute in essential hydrocarbon and cholesterol synthesis, which are important components of eggs. Markable difference in longevity showed impacts of animals unable to utilize certain lipids in *de novo* pathway, since feeding on a regular diet saturated in different fatty acids did not benefit life span. Difficulty in processing fatty acids ingested from diet may also have dietary restriction effects on these animals. Since they are unable to further convert these fatty acid into forms that will benefit physiological functions, these animals may not be able to use these fatty acid as energy source or storage. Previous studies with SCD-1 knocked out mice, with very limited fat body even being fed high fat diet, also provided support for possible dietary restriction function of delta-9 desaturases.

III. Lipid analysis.

Although lipid profile results showed no significant changes between control and the two knocked-down strains, this can result from a high concentration of fatty acids from regular diet. (Fig 19) Having disrupted delta-9 desaturases, the animals have limitations on metabolizing these fatty acids and utilizing them de novo. However, fatty acids do get stored within the animals. (1)

When rearing on minimum diet, lipid contents of larvae decrease noticeably. Since low expression of either delta-9 desaturases hinders lipid metabolism, and these animals are not consuming much lipid from food media; the concentrations in lipids is low, even though they still maintain a somewhat similar compositions in comparison to control group. (Fig. 22 and 23)

IV. Rearing on minimum diet.

To further understand the importance of delta-9 desaturases in *Drosophila* development, we tried rearing both: control and knocked-down strains; on minimum diet. It is observed that both Desat 1 and Desat 2 knocked-down strain do not survived pass second instar. This can result from hindrance of making necessary precursor for various phospholipids, which are essential for formation of cell membranes.

However, when oleic acid was added to minimum diet, survival of larvae increased from zero to close to twenty percent. This resulted from some utilization of oleic acid in lipid metabolism pathways since these animals were knock-down and not knocked-out. Previous studies in mice showed that with SCD-1 knocked out, feeding essential fatty acid will not rescue abnormal phenotypes. (6)

V. Future studies

Further experiments to investigate TAG levels in eggs of different KD and control groups may assist in understanding the energy invested in offspring productions. This can aid investigation of impacts on life span under reproduction pressure when delta-9 desaturases are disrupted. Possible studies also include activity measurements along with rate of oxygen consumptions to understand the relationships between delta-9 desaturases and energy metabolism in *Drosophila*.

APPENDIX I

REGULAR FOOD

(CORNMEAL-MOLASSES-AGAR FOOD)

**Before attempting to make this food on your own,

ask someone who knows how to help you !!!

INGREDIENTS

Step	Ingredient	1.5 Batch	1.25 Batch	1 Batch	3/4 Batch	1/2 Batch	1/4 Batch
1	Distilled Water	9 L	7.5 L	6 L	4.5 L	3 L	1.5 L
1	Agar	67 g	56 g	45 g	34 g	22 g	11 g
2	Molasses	525 mL	440 mL	350 mL	250 mL	175 mL	90 mL
3	Yellow Cornmeal	900 g	750 g	600 g	450 g	300 g	150 g
4	Torula Yeast	750 g	625 g	500 g	375 g	250 g	125 g
5	Distilled Water	~1.5 L	~1.25 L	~1 L	~750 mL	~500 mL	~250 mL
6	95% Ethanol	150 mL	125 mL	100 mL	75 mL	50 mL	25 mL
6	Tegosept (p-hydrobenzoic acid methyl ester)	30 g	25 g	20 g	15 g	10 g	5 g
7	Propionic Acid	60 mL	50 mL	40 mL	30 mL	20 mL	10 mL
	Yield	1200 vials 225 bottles	1000 vials 180 bottles	800 vials 150 bottles	600 vials 110 bottles	400 vials 75 bottles	200 vials 30 bottles 12 petri plates

Directions

Step 1:

In a large aluminum pot, add the distilled water. While mixing, add in the agar. Once the agar is stirred into the water, place the pot on the stand over the Bunsen burner. Stir continuously using a wire whisk. **CRITICAL STEP: Stir until the mixture comes to a FULL ROLLING BOIL! If the mixture does not boil, it will not set up and cannot be used.**

Step 2:

Once the mixture has come to a full rolling boil, add the molasses and stir. Once the molasses is in solution, remove it from heat.

Step 3:

After removing the mixture from the heat, **gradually** add the cornmeal while stirring continuously. **If the cornmeal is not added gradually, it will form clumps and you will have to start over.**

Step 4:

Once the cornmeal is in the solution, add the yeast while stirring continuously.

Step 5:

Once the yeast is in the solution, add enough water to bring the food to the correct consistency. Once you have been taught how to make food, you will know what this consistency is. (It looks like thin Cream of Wheat.) Remember to keep stirring.

Step 6: (ANTI-FUNGAL SOLUTION)

In a graduated cylinder, add the ethanol and the Tegosept. Make sure the Tegosept goes into the solution. **This solution can be added to the food when the food has cooled to less than 65°C.**

Step 7:

Once the antifungal solution has been added to the food, you can add the propionic acid. The food is now ready for pouring.

APPENDIX II

Lipid Extraction according to Bligh and Dyer

1. Add 1 ml water to each sample, vortex, to resuspend tissue, cells, etc. If using tissues, you will want to use a dounce homogenizer to disrupt all of the tissue. Following disruption, transfer tissue homogenate to a new glass tube that can be spun up to 3,000 rpm. (Internal lipid standard can be add here if using GC).
2. Add 3.75 ml CHCL3:CH3OH(1:2) and vortex over a 30 minute period with agitation; make sure the samples do not splash out.
    - (2 ml CHCL3 + 0.05% BHT + 4ml CH3OH +0.05% BHT)
3. Pellet cellular debris by spinning at 3,000 rpm for 5 minutes.
4. Transfer entire sample to a new glass tube; do not transfer pelleted debris.
5. Add 1.25 ml water and 1.25 ml CHCL3 to each sample (biphasic solution should result).
6. Vortex and spin samples at 3,000 rpm for 5 minutes.
7. Remove the upper aqueous/methanolic phase and discard; transfer the lower CHCL3 phase with the lipids to new tube.
    - The CHCL3 phase should constitute ~2 ml total volume.
8. Dry down solvent under extra dry nitrogen gas.
    - If solvent does not dissolve, add 1 or 2 ml CHCL3, remove aqueous layer, and dry again until only solid clear layer remains at bottom.

APPENDIX III

GC fatty acid methyl ester (FAME's) analysis-acidified methanol

1. Take a portion of the lipid extract and dry down the solvent at 50°C under a stream of nitrogen.
2. Resuspend lipid in 250µL toluene and 500µL 1% H2SO4 in methanol.
3. Cap under nitrogen and place on heating block at 50°C overnight.
4. Cool samples to room temp.
5. Add 1.25mL 5% NaCL and vortex.
6. Add 1.25mL hexane, vortex and centrifuge at 1500 rpm for 5 minutes to separate phases.
7. Collect upper organic phase and transfer to a clean 13X100mm glass tube.
8. Repeat hexane extraction in step 6 and combine with first hexane extractions. Dispose of lower phase.
9. Add 1mL 2% potassium bicarbonate to combined hexane extractuions, vortex and allow phases to separate.
10. Pass upper phase over ~0.5 grams sodium sulfate (in a packed glass pasture pipette) and collect in a clean 13X100 mm glass tube.
11. Dry under a stream of nitrogen at 50°C.
12. Resuspend FAME's in 1 mL methyl acetate and transfer to GC vials and cap for analysis. Samples can be stored at -20°C.