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Nutritional quality during development alters insulin-like peptides expression and physiology of the adult yellow fever mosquito, *Aedes aegypti*

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Abstract:

Mosquitoes have distinct developmental and adult life history, and the vectorial capacity of females has been shown to be affected by the larval nutritional environment. However, little is known about the effect of developmental nutrition on insulin-signaling and nutrients storage. In this study, we used *Aedes aegypti*, the yellow fever mosquito, to determine whether larval nutrition affects insulin gene expression. We also determined the traits regulated by insulin signaling, such as stored-nutrients levels and fecundity. We raised mosquito larvae on two different diets, containing either high protein or high carbohydrates. Development on a high-carbohydrate diet resulted in several life-history phenotypes indicative of suboptimal conditions, including increased developmental time and decreased fecundity. Additionally, our data showed that insulin transcript levels are affected by a high-carbohydrate diet during development. Females, not males, reared on high-carbohydrate diets had much higher transcript levels of insulin-like peptide 3 (ILP3), a mosquito equivalent of human insulin, and these females more readily stored sugar from the meal into lipids. We also found that AaILP4, not AaILP3, transcript levels were much higher in the males after a sugar meal, suggesting sex-specific differences in insulin-signaling pathway. Our findings suggest a conserved mechanism of carbohydrate-mediated hyperinsulinemia in animals.

Keywords: insulin-signaling, insulin receptor, Aedes aegypti, mosquitoes, insulin, insulin-like peptides, nutrition, hyperinsulinemia, larval-diet, metabolic reserves

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Abbreviations:

ILP: insulin-like peptides

Dilp: Drosophila insulin-like peptides

IR: insulin receptor

L1-4: Larval instars 1-4

RT-PCR: Reverse transcription Polymerase Chain Reaction

qRT-PCR: quantitative Reverse transcription Polymerase Chain Reaction

1. Introduction

A variety of environmental factors such as light, temperature, and nutrient availability affect the development and physiology of animals. Adaption to changes in nutrition often involves hormonal regulation and metabolic homeostasis [1]. In mammals, insulin and glucagon are key systemic regulators which maintain circulating glucose levels during fluctuating nutritional conditions [2]. Similarly, hemolymph carbohydrate levels in insects are regulated by the action of two endocrine hormone families: insulin like peptides (ILPs), which are structurally and functionally analogous to vertebrate insulin, and adipokinetic hormone, a functional analog of vertebrate glucagon [3-5].

Insulin/Insulin-like growth factor signaling in the yellow fever mosquito, *Aedes aegypti*, is mediated by eight ILPs (AaILPs). AaILP1, AaILP3, and AaILP8 predominantly express in the adult brain, AaILP4 and AaILP7 express in both brain and ovaries, and AaILP2, AaILP5, and AaILP6 are ubiquitously expressed in most adult tissues [6]. AaILP3 has been shown to bind to and signal through a tyrosine kinase receptor, the mosquito insulin/Insulin growth factor receptor (MIR), and it is predicted that more than one ILP is capable of binding to this common MIR. AaILP3 modulates circulating carbohydrate, glycogen, and lipid levels [7, 8]. AaILPs serve additional roles in coordinating blood meal digestion and yolk deposition in adult female *Ae. aegypti* [9]. Ecdysteroids synthesis by ovaries requires five times higher AaILP4 levels compared to AaILP3, and unlike AaILP3, it does not affect circulating carbohydrates, stored glycogen, and lipid levels [10]. Additionally, AaILP3 in *Ae. aegypti*, not in *Ae. atropalpus*, regulated ecdysteroids synthesis by ovaries and yolk synthesis in eggs [11]. AaILP7 and AaILP8 have recently been shown to regulate glycogen and lipid metabolism and ovarian development [12]. AaILP7 modulates lipid deposition before a blood meal for egg development, and AaILP8 appears to modulate lipid mobilization. The glycogen levels exhibited the opposite

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trends in these mosquitoes, which indicate that AaILP7 and AaLP8 are also involved in the regulation of sugar [12]. Functions of the other four ILPs in *Ae. aegypti* have not yet been explored.

Nutrient quantity and quality affect the expression of ILPs in other insects. In the honey bee, *Apis melifera,* out of two ILP genes, *AmILP1* and *AmILP2*, *AmILP1* was most affected by diet composition and was most expressed in high protein diet [13]. Most species of mosquito larvae are non-selective filter feeders of organic particles suspended in water and of micro-organisms such as bacteria, viruses, protozoans and fungi [14]. Larval instars 3 (L3) and L4 feed vigorously and store nutrients for the non-feeding pupal stage; therefore, information on nutrient signaling is critical in this developmental period. Given the complex compensatory expression of ILPs, we hypothesized that the ILPs will have different expression profiles in *Ae. aegypti* reared on different diets. To test this hypothesis, we measured the effects of different larval diet composition on ILPs expression. We also studied the impacts on subsequent adults eclosed from the larvae fed on diets that had similar caloric values but varied by protein:carbohydrate ratio. Thirdly, we measured levels of circulating carbohydrates, stored glycogen, lipids, proteins, and life history traits such as developmental time, body size, lifespan, and fecundity. Our data suggest that diets consisting of different protein: carbohydrate ratios result in differential expression of AaILPs. The high protein diet resulted in shorter developmental time, higher lipid levels, and more fecund females compared to high carbohydrate diet. There was no significant difference in body size in males whereas females of protein-rich diet were significantly bigger than carbohydrate-rich diet. Adults eclosed from the protein-rich diet fed larvae had longer lifespan. Our results also suggest differences in nutrient metabolism between males and females; females with a diet low in protein (high in carbohydrates) during development tend to convert the sugar meal into fat more readily than males. Developmental nutrients did not have any significant effect on stored metabolites in males, suggesting the higher need for lipid in females, presumably for host-seeking and reproduction.

2. Materials and Methods:

2.1 Mosquitoes

The UGAL strain of *Ae. aegypti* was used in all experiments. All stages were maintained in an insectary under a 16 h light: 8 h dark photoperiod and a temperature of 27°C and 80% relative humidity [11]. Eggs were hatched overnight. Larvae were reared in plastic containers. 150 first instar larvae were counted and reared in 500 ml de-ionized water. Equal amounts (by

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weight) of standard fish food (Tetramin®, Melle, Germany) or gerbil food (Small World®, St. Louis, USA) were provided to larvae every day. The fish food (protein rich) was composed of ≥46% protein, ≥12% fat, ≤3% carbohydrates; the gerbil food (carbohydrate rich) had ≤20% protein, ≥10% fat and ≥20% carbohydrates. The standard larval diet was as follows: days 1 and 2 post larval emergence – 150 mg diet/pan, days 3 and 4 – 300 mg/pan, day 5 and 6– 600 mg/pan, day7 - no diet (start pupation day 8). Most mosquitoes on a high-carbohydrate diet (gerbil food) pupated by day ten. Pupae were collected from the rearing pans and kept in adult cages for emergence. Adults were provided continuously with water and a 10% sucrose solution. Females were blood fed on day 4 post eclosion.

2.2 Quantitative expression analysis of ILPs by real time RT-PCR

The quantitative expression profiles of AaILPs were conducted in fourth instar larvae and adult male and female mosquitos reared on a fish and gerbil food regime (hereafter, protein-rich and carbohydrate-rich diets, respectively). Five L4 were pooled for RNA extraction. Adult mosquitoes (10 mosquitoes per sample) were collected for RNA isolation at two time points: 1) 24 h post eclosion without providing any sugar, and 2) fed on 10% sugar solution for 10 minutes, samples collected one hour post sugar meal. Total RNA was isolated from a pool of 10 individuals per replicate (three replicates per time point per cohort) using TRIzol reagent (Invitrogen, Waltham, MA, USA). 5 µg of total RNA was used for DNase treatment (Sigma, St. Louis, MO, USA) according to the manufacturers' protocol. DNase-treated RNA samples were re-purified with Trizol. 1 µg of DNase treated RNA was used for cDNA synthesis with iScript reverse transcription supermix (BioRad, Hercules, CA, USA). cDNA was diluted 10x before using as a template in qRT-PCR experiments. qRT-PCR was performed on CFX touch Real-Time PCR Detection system (BioRad, Hercules, CA, USA), using SYBR green master mix (BioRad). Sequences of specific primers for AaILPs, MIR, and a housekeeping gene, ribosomal protein s7, are listed (Table 2). All reactions were performed with 120s at 95 °C, followed by 40 cycles of 10s at 95 °C, 15s at 58 °C, and 15s at 72 °C, and melt curve was analyzed at 70-95 °C. Relative expression was calculated using $2^{-\Delta\Delta Ct}$ method, where protein rich (fish-food) diet fed mosquitoes were considered control and expression of ILPs and MIR in carbohydrate-rich (gerbil) diet fed mosquitoes was calculated relative to the control levels. Experiments were replicated four times with different mosquito cohorts.

2.3 Mosquito physiology bioassays

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2.3.1 Developmental time: Number of larvae molted to L2, L3, L4, and pupal stages were counted daily. Exuviate and dead larvae were removed each day from the pans. Pupae were collected and transferred to emergence cages. The experiment was replicated five times with different cohorts.

2.3.2 Fecundity: Five females per replicate per treatment were kept individually in small cages made out of specimen cups, lined with a moist paper towel. The number of eggs deposited by each female was counted 5 days post blood meal (PBM). Females that did not deposit eggs were dissected to examine the ovaries for developed oocytes. The experiment was replicated five times with different mosquito cohorts.

2.3.3 Metabolic Assays: Trehalose, glycogen, lipid, and protein levels were quantified 1) 24 h post eclosion provided with deionized water only without a sugar meal (teneral reserves), and 2) fed on 10% sucrose solution for 4 h, separated from the unfed and 20 h post sugar meal (allocation of nutrients from a sugar meal for storage). Sugar uptake was confirmed by looking at the mosquitoes under the microscope for swollen abdomens and blue color from food dye in the sugar solution. Sugar-fed mosquitoes were maintained for 20 h before assays. Samples were processed for micro-separation of metabolites from both males and females. In brief, two adults per sample were collected in 100 µl of Na_2SO_4 and 200 µl of methanol in microfuge tubes where they were either homogenized or stored at −80° C. These samples were further processed and compartmentalized in trehalose (circulating carbohydrates), glycogen, and lipids as previously described [7, 8]. Levels of trehalose and glycogen were estimated with Anthrone assay and lipids with Vanillin assay [8]. For protein assay, two adults per sample were collected in phosphate buffer saline with protease inhibitor (PBS+ PI). Total proteins were estimated with Bradford assay.

2.4 Data analysis

All data were analyzed using the GraphPad Prism 7 software (La Jolla, CA, USA). AaILP data was analyzed by unpaired t-test. Mosquito teneral reserves data were examined by one-way ANOVA followed by the Tukey's multiple comparisons test with each treatment serving as the independent variable. The effect of diet on the number of eggs deposited was examined by t-test.

3. Results

3.1 ILP expression changes in mosquitoes reared on different diets

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Ae. aegypti larvae hatched from the same egg cohort were fed on equal amounts (by weight) of either protein-rich or carbohydrate-rich diet from emergence to pupal molt. Relative ILP transcript expression was normalized to high-protein diet fed mosquitoes for each group. In L4 larvae, AaILP8 was highly expressed, whereas AaILP7 expression was low (Fig. 1). In adult males, AaILP4 had higher expression, whereas in females AaILP3 and AaILP6 transcripts were significantly higher. AaILP5 expression was low in both adult males and females, whereas AaILP4 and AaILP7 were less expressed in females (Fig. 1). AaILP4 was expressed in adults but not in the larval stages in either food group. AaILP2 was barely detectable in any of the life stages tested, and no changes were detected (data not shown).

3.2 Protein rich diet resulted in shorter developmental time

The protein-rich diet fed larvae completed their development to pupae in 7-8 days, which is standard in our laboratory and other laboratories [15]. However, the carbohydrate-rich diet fed larvae showed delayed development. On average, pupal emergence was delayed by 2 days. Developmental delay started from L2-L3 molt (Table 1).

3.3 Levels of circulating carbohydrates, glycogen, and lipids altered in adults from different diet fed larvae, whereas protein levels remained constant

Levels of circulating carbohydrates (trehalose) and stored nutrients (glycogen, lipids, and protein) were measured in adult females and males 24 h (before sugar meal) and 48 h (after a sugar meal) post-eclosion. In newly eclosed, unfed males, levels of glycogen and lipid were equal in both protein-rich and carbohydrate-rich diet fed animals; however, trehalose and protein levels were significantly lower in males emerged from the larvae reared on a carbohydrate- rich diet compared to those fed on protein-rich diet (Figs. 2, 3). In sugar-fed males, levels of all metabolites were equal in both diets. In females, the effect of larval diets was more pronounced. In newly eclosed, unfed females, lipid levels were significantly lower in the carbohydrate-rich diet group, whereas glycogen, trehalose, and protein levels were equal in both diets (Figs. 2, 3). After a sugar meal, levels of glycogen, trehalose, and lipids increased significantly in sugar-fed females raised on protein-rich diet compared to the unfed control, whereas protein levels did not change (Fig. 3). In the carbohydrate-rich diet samples, the sugar meal had no significant effect on the levels of glycogen, trehalose, and protein, whereas lipid levels increased significantly compared to unfed females. Except for protein, metabolite levels were significantly higher in protein-rich diet females compared to the carbohydrate-rich diet females after a sugar meal (Fig. 2).

3.4 Protein-rich diet resulted in higher fecundity

Females from larvae reared on a carbohydrate-rich diet had decreased fecundity. The protein-rich diet group deposited an average 82 eggs, whereas the carbohydrate-rich group deposited only 20 eggs on average (Fig. 4).

4. Discussion:

Our data suggest that developing on a carbohydrate-rich diet alters mosquito insulin-like peptides levels and affects nutrient storage, larval development time, and fecundity. Nutrition is a primary determinant of life span and reproductive capacity in all organisms, from yeast to humans [16-18], and its relationship to life history has been studied extensively. Developmental nutrition can have far-reaching effects on adult traits [19-23]. A wide array of insect literature has found that calorically poor food quality during development leads to increased development time, decreased adult weight [23-26], and reduced fecundity. However, the effect of nutrient quality and the relationship with ILPs that maintain nutrient homeostasis has not yet gained much attention.

Ae. aegypti ILPs 1–8 have been identified and named mostly through their sequence homology to the *Drosophila melanogaster* and mammalian insulin and the typical B-C-A domain structure as observed in mammalian insulin [4](Gronke et al., 2010). The *Ae. aegypti* ILPs1-7 (AaILPs) are othologs of *D. melanogaster* dilps 1-7, with the exception that *Ae. aegypti* ILP2 and AaILP3 are closer orthologs of dilp3 and dilp2, respectively (personal observation; sequence alignment). Dilp8 was recently identified based on the conserved cysteines [27, 28].

Our data here suggest that manipulating food quality during larval development has significant effects on ILPs expression. In *D. melanogaster*, starvation reduced larval dilp3 and dilp5 expression [29], whereas starvation increased dilp6 expression in larvae and adults [30, 31]. When maintained on yeast-restricted or diluted diets, dilp5 was reduced in both larval and adult flies [32-34]. We did not observe any difference in the levels of AaILPs 3, 5, and 6 in larvae; however, AaILP1 was upregulated and AaILP7 was downregulated in carbohydrate-rich diet fed mosquitoes, suggesting differences between *D. melanogaster* and *Ae. aegypti* insulin signaling. AaILP8 was most significantly upregulated in L4. In *D. melanogaster*, Dilp8 transcript levels peak during the transition from larval instars and have been shown to control the timing of larval molts, ensuring that animals do not progress to the next developmental stage before adequate growth has happened [28]. Higher transcript levels of AaILP8 in the L4 might have a

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similar function in regulating molt to pupal stage. The difference in AaILP8 peak in carbohydrate-rich vs protein rich diet may be due to the difference in the timing of L4s since carbohydrate-rich diet fed mosquitoes took two to three extra days to become L4, and the samples were collected on different days. The synthetic *Ae. aegypti* ILP8 had no effect on adult nutrient metabolism (unpublished data, Gulia-Nuss), which further suggests a different role of this ILP; however, a recent study suggested the role of AaILP8 in lipid mobilization after a blood meal [12].

Feeding *D. melanogaster* adults a diet comprised of different protein-to-carbohydrate ratios at variable caloric concentrations resulted in higher dilp2 expression in high- carbohydrate diets regardless of total caloric value, whereas dilp5 level increased with protein level [35]. Our data follows the same trend because AaILP3 levels (dilp2 ortholog) increased and AaILP5 levels decreased (i.e. increased transcript levels in protein rich) in adults in the carbohydrate-rich diet group, suggesting a functional similarity with dilp3 and dilp5. AaILP3 has been shown to be mammalian insulin equivalent in both structure and function [7]. A single injection of synthetic ILP3 in sugar-fed, decapitated *Ae. aegypti* females was able to restore trehalose, glycogen, and lipid levels similar to the intact control [7]. Our data supports these previous findings and further strengthens the role of AaILP3 in adult female nutrient metabolism. The function of ILP5 has not been studied in *Ae. aegypti* and is worth exploring.

AaILP4 does not bind directly to the MIR and had no effect on nutrient metabolism in sugar-fed females [10]. AaILP4 was expressed in both male and female adults but not in the larval stages in either food group. Higher expression of AaILP4 in males is an interesting finding because this ILP was previously considered female-specific [10]. The tissue expression of AaILP4 in males might shed light on the possible functions of this ILP. *Ae. aegypti* ILP6 is believed to be an insulin growth factor because of a shorter C chain and no cleavage site [6]. Increased expression of this ILP in carbohydrate-rich diet fed females also needs further exploration. Unlike *D. melanogaster*, MIR expression was constant in all mosquito life stages, suggesting regulation at the ligand (ILP) level.

Our data show that manipulating food quality during larval development has significant effects on adult mosquito physiology. Mosquitoes reared on equal amounts (w/w) of protein-rich food lived longer (Supplementary Fig. 1) and deposited more eggs compared to those reared on high-carbohydrate diets. The effect of different larval diets was also apparent in levels of stored and circulating nutrients in adults. Male mosquito eclosed from the carbohydrate-rich diet had lower levels of trehalose; however, glycogen, lipid, and protein levels were equal.

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Energetic needs change dramatically between life stages in holometabolous insects, particularly in female insects, since nutrient investment into eggs constitutes a major expenditure of energy [36]. Therefore, most studies have focused on female nutrient allocation. The nutritional environment experienced by insect larvae strongly influences female fitness-related traits such as body size, teneral metabolic reserves, and fecundity [37-39]. In *A. aegypti*, approximately 80% of lipids found in eggs are derived from regular sugar meals before blood feeding [40]. Because egg development is dependent on a female's reserve of nutrients, it is important that she restrict oogenesis until sufficient nutrients are available or risk starvation. Presumably, the endocrine and nervous systems monitor these reserves and regulate physiological, developmental, and behavioral processes that rely on these reserves accordingly. In our study, there was no difference in body size in males whereas females of protein-rich diet were significantly bigger than carbohydrate-rich diet (Supplementary Fig. 2). Adults eclosed from the protein-rich diet fed larvae had longer lifespan. Females eclosed from a carbohydrate-rich diet were more susceptible to dying earlier than males (only 30% males on a protein-rich diet survived longer than those on a carbohydrate-rich diet, whereas this difference was 50% in females) (Supplementary Fig. 1). In *Anopheles stephensi*, larvae reared under a reduced diet had increased adult mortality [41].

Our results also suggest differences in nutrient metabolism between males and females. When fed carbohydrate-rich food during development, adult females, but not males, converted the sugar meal into lipid reserves more readily. It is also notable that the carbohydrate-rich group females had lower lipid levels at eclosion, and this increase in lipids post sugar meal might be required for maintenance of homeostasis. In *Ae. sollicitans*, when the adults were maintained on sugar meal only, females had higher levels of fat than males [42]. When females were starved until no fat remained and then fed on sugar, fat levels increased, and newly synthesized fat appeared, entirely made from sugars [43], further suggesting that optimum fat levels are needed for females to survive. In mammals, high carbohydrate and low protein intake is positively associated with hyperlipidemia. Low-carbohydrate, high-protein, low-energy diets are widely used in weight management programs. A growing body of evidence suggests that the high-protein, low-carbohydrate diets improve hyperinsulinemia [44]. These results suggest a similar control mechanism of metabolic stores and insulin signaling in both vertebrates and invertebrates. Analyzing how nutrient composition associates with the expression of ILPs reveals that nutritional status is modulated by different combinations of ILPs in males and female. Our further experiments with CRISPR-Cas9 knockouts will permit deeper examination of the roles of each specific ILP in mosquito nutrient homeostasis. Together, these observations

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suggest that developmental diets can influence the insulin-signaling mediated life-history traits during adult life, and these affects vary in males and females, suggesting sex-specific differences in nutrient regulation.

Competing Interests: The authors do not have any competing interests in the manuscript.

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Tables:

Table 1: Carbohydrate rich larval diet results in delayed development. Larvae were hatched from the same batch of eggs and reared under same conditions. Both sets were given equal amounts (by weight) of food. Carbohydrate rich diet fed larvae received extra food during prolonged developmental period.

Diet	**Time in hours**				**Total developmental time (days)**
	L1 to L2	**L2 to L3**	**L3 to L4**	**L4 to pupae**	
Protein-rich	38 ±5	44±4	48±5	48±4	6.7 -8.2
Carbohydrate-rich	38±4	50±6	64±6	84±8	8.8- 10.8

Table 2: Primer sequences for real-time RT-PCR. Sequences for all insulin-like peptides (ILPs), insulin receptor, and the housekeeping gene, ribosomal protein S7, are noted.

Primer Name	**Primer sequence**
ILP1 Fwd	5’- ACTGGTTTGCAACAGCTACC-3’
ILP1 Rev	5’- TCCAGGTCCTGTTTGATCTC-3’
ILP2 Fwd	5’- CATCACCGCTCAGAATACCT-3’
ILP2 Rev	5’- AGAACGGAAAACCGTGACTA-3’
ILP3 Fwd	5’- ACCAACTTGCGAGTATCGAG-3’
ILP3 Rev	5’- TGTACTACGGTTCCGACCAT-3’
ILP3 Fwd	5’- TACTCGAAGCACGACCCTAT-3’
ILP3 Rev	5’- GGCAACATTCCTCTACGATG-3’
ILP3 Fwd	5’- CTAATCCGGCACCTTTACTG-3’
ILP3 Rev	5’- AAGGGTAGCGCATTAGCAC-3’
ILP3 Fwd	5’- GAGCAAATCCACAACTCCAG-3’
ILP3 Rev	5’- GCACAGTTCCAAATTCCATC-3’
ILP7 Fwd	5’- GCGCCAACTATGACAAAACT-3’
ILP3 Rev	5’- AGGGTTTGTAGCAACAGTCG-3’
ILP8 Fwd	5’- AGGGCCATTCTACAAGCTCT-3’
ILP3 Rev	5’- AGGAATGTTTCTCCGTGTCC-3’
Insulin receptor Fwd	5’- AATGGTTACCGCCACTGAAG-3’
Insulin receptor Rev	5’- GCACTGATCCGCAGTACAGA-3’
Ribosomal protein S7 Fwd	5’- ACCGCCGTCTACGATGCCA-3’
Ribosomal protein S7 Rev	5’- ATGGTGGTCTGCTGGTTCTT-3’

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Figure Legends:

Fig. 1: Transcript levels of insulin-like peptides in adult mosquitoes from larvae reared on different diets. ILPs transcript levels were determined by qRT-PCR in larval instar four (L4, top) male (middle) and female (bottom) adults eclosed from the larvae reared either on protein-rich or carbohydrate-rich diet. Protein-rich diet fed samples were used as a control for the relative transcript expression determination. The horizontal line on the graphs represents transcript levels in the protein-rich samples (control). The vertical bars represent the levels of ILPs in carbohydrate-rich diet fed (experimental) samples relative to the control. Unpaired t-test was used for significance analysis. Experiments were replicated four times with different cohorts. Each cohort consisted of three replicates. n=12.

Fig. 2: Effect of larval diets on adult trehalose, glycogen, and lipid levels. Two 24 h old, unfed adult female and male mosquitoes per sample from the control (high protein) and experimental (high carbohydrate) group were used to assess teneral nutrient reserves. Mosquitoes from same cohort were sugar-fed for 4h and then processed 20h later to assess differences in nutrient storage in control and experiment groups. Levels of glycogen (top), trehalose (middle), and lipids (bottom) were determined in females (first four vertical bars) and males (last four vertical bars) in both protein-rich (black bars) and carbohydrate-rich (gray bars) diet samples. One-way ANOVA followed by Tukey's multiple comparison was used. Glycogen: F (D_{Fn}, D_{Fd}): (7, 16) = 7.819, $P<0.0003$; Trehalose: F (7, 16) = 26.89, $P<0.0001$; Lipids: F (7, 16) = 23.19, $P<0.0001$. Experiments were replicated thrice with different cohorts. P=protein-rich diet fed; C= carbohydrate-rich diet fed; UF=unfed; SF= sugar-fed.

Fig. 3: Effect of larval diets on adult protein levels. Two 24 h old, unfed adult female and male mosquitoes per sample from the control (high protein) and experimental (high carbohydrate) group were used to assess teneral nutrient reserves. Mosquitoes from the same cohort were sugar-fed for 4h and then processed 20h later to assess differences in nutrient storage in control and experiment groups (N=6). Protein levels in males (top) and females (bottom) were determined in both protein-rich (black bars) and carbohydrate-rich (gray bars) diet samples. One-way ANOVA followed by Tukey's multiple comparison was used. Male: F (DFn, DFd): (3, 20) = 4.642, P=0.0128; Female: F (3, 20) = 2.182, P=0.1219

Fig. 4: Effect of larval diets on fecundity. A subset of five females per treatment were kept individually in small containers lined with moist paper towels to provide an oviposition surface.

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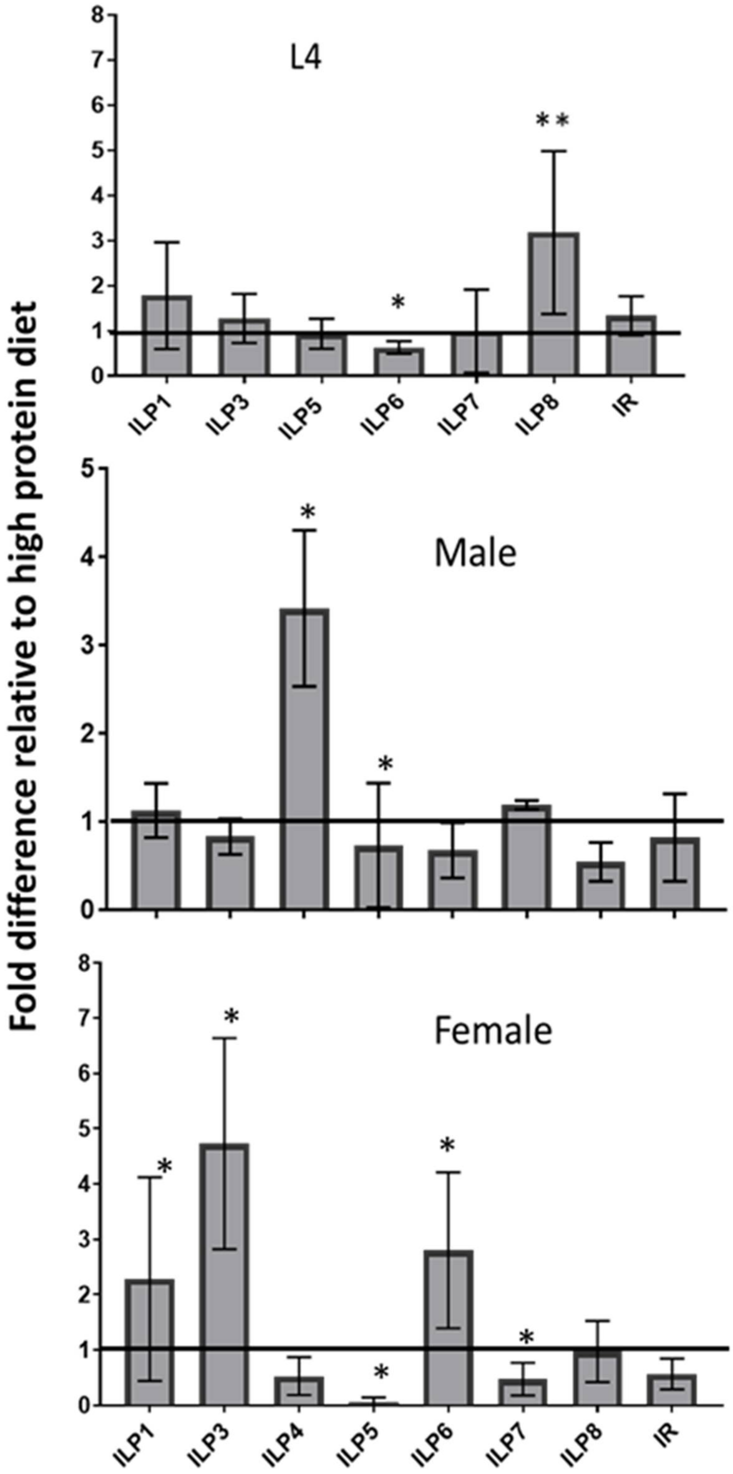
The number of eggs deposited by individual females was counted five days post-blood meal. Mean ±Standard error were plotted. Data were analyzed by t-test (t=9.599 df=18), P<0.0001. Experiments were replicated thrice with different cohorts. N=15

Figure 1


Fold difference relative to high protein diet
L4
8
7
6
5
4
3
2
1
0
**
*
ILP1
ILP3
ILP5
ILP6
ILP7
ILP8
IR
Male
5
4
3
2
1
0
*
*
Female
8
7
6
5
4
3
2
1
0
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*
*
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ILP1
ILP3
ILP4
ILP5
ILP6
ILP7
ILP8
IR

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Figure 2

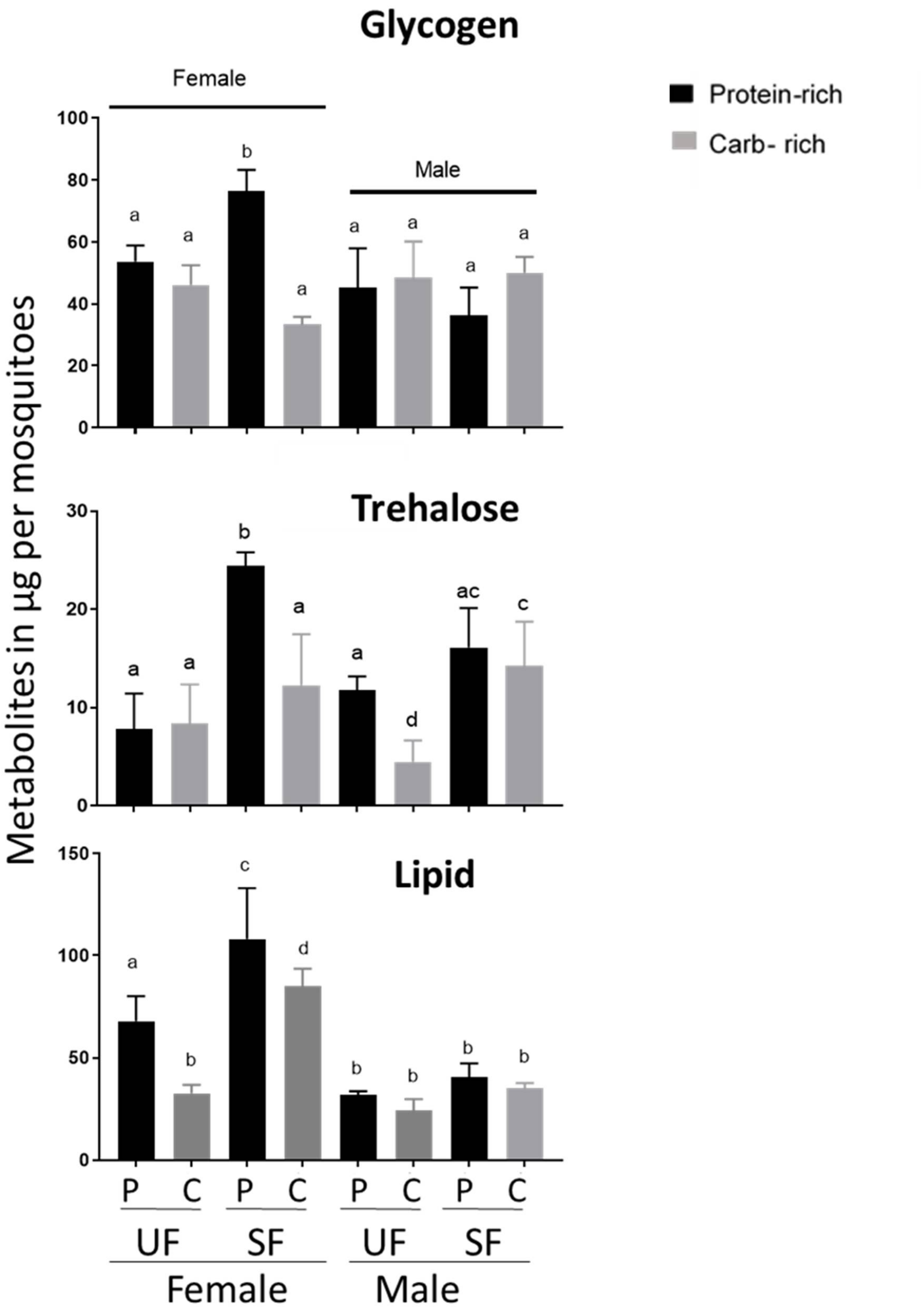

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Figure 3

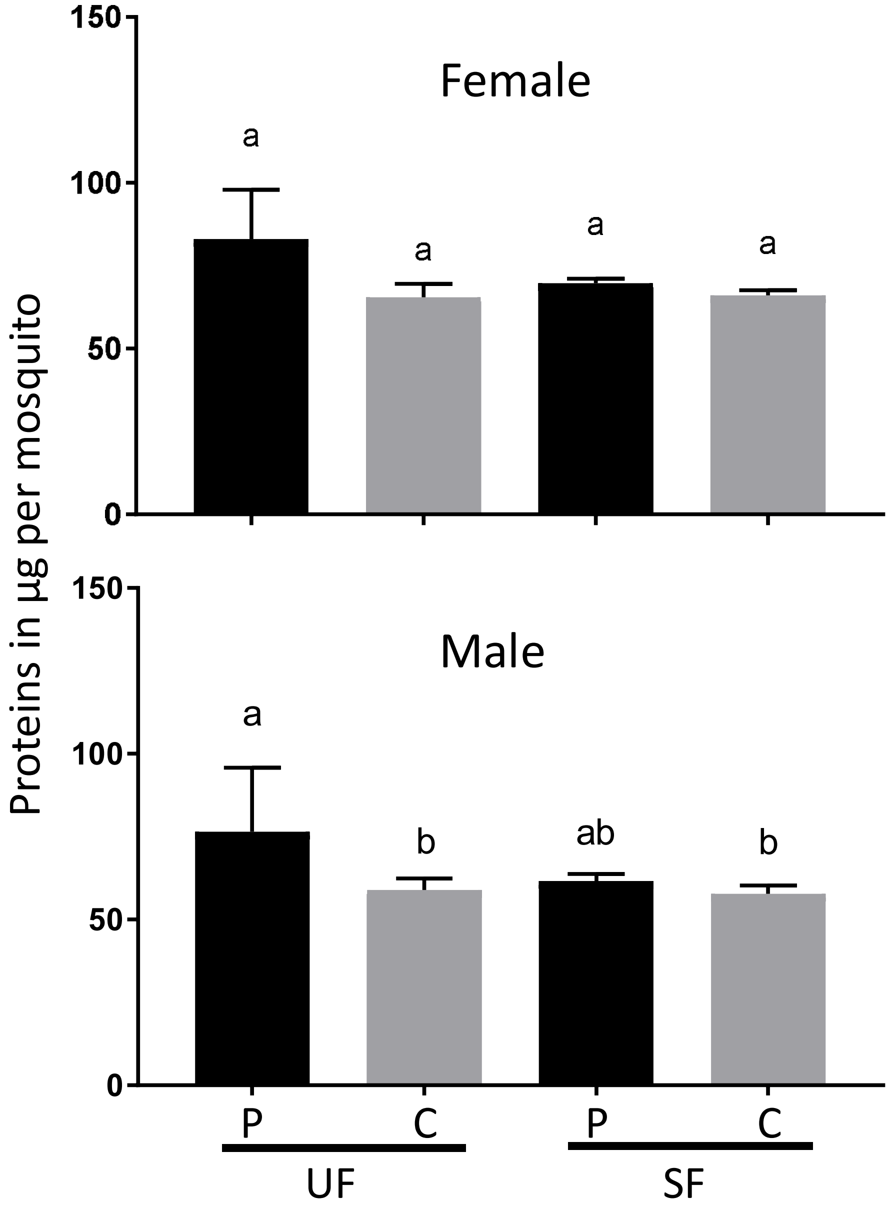

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Figure 4