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Journal of Insect Physiology 58 (2012) 1007–1019

Contents lists available at SciVerse ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

The fate of follicles after a blood meal is dependent on previtellogenic nutrition and juvenile hormone in *Aedes aegypti*

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ARTICLE INFO

Article history:
Received 23 February 2012
Received in revised form 30 April 2012
Accepted 3 May 2012
Available online 22 May 2012

Keywords:
Juvenile Hormone
Mosquito
Oosorption
Sugar feeding
Reproduction
Vitellogenesis

ABSTRACT

Juvenile hormone (JH) mediates the relationship between fecundity and nutrition during the gonotrophic cycle of the mosquito in three ways: (1) by regulating initial previtellogenic development, (2) by mediating previtellogenic resorption of follicles and (3) by altering intrinsic previtellogenic follicle "quality", physiology, and competitiveness thereby predetermining the fate of follicles after a blood meal. To support a role for JH in mediating the response of ovarian follicles after a blood meal, we explored three main questions: (1) Do changes in nutrition during the previtellogenic resting stage lead to relevant biochemical and molecular changes in the previtellogenic ovary? (2) Do hormonal manipulations during the previtellogenic resting stage lead to the same biochemical and molecular changes? (3) Does nutrition and hormones during the previtellogenic resting stage affect vitellogenic resorption and reproductive output? We examined the accumulation of neutral lipids in the previtellogenic ovary as well as the previtellogenic expression of genes integral to endocytosis and oocyte development such as the: vitellogenin receptor (*Aa*VgR), lipophorin receptor (*Aa*LpRov), heavy-chain clathrin (*Aa*CHC), and ribosomal protein L32 (rpL32) under various previtellogenic nutritional and hormonal conditions. mRNA abundance and neutral lipid content increased within the previtellogenic ovary as previtellogenic mosquitoes were offered increasing sucrose concentrations. Methoprene application mimicked the effect of offering the highest sucrose concentrations on mRNA abundance and lipid accumulation in the previtellogenic ovary. These same nutritional and hormonal manipulations altered the extent of vitellogenic resorption. Mosquitoes offered 20% sucrose during the previtellogenic resting stage had nearly 3 times less vitellogenic resorption than mosquitoes offered 3% sucrose despite taking smaller blood meals and developed ~10% more eggs during the first gonotrophic cycle. Mosquitoes treated with JH III during the previtellogenic resting stage and then offered a blood meal had a ~40% reduction in the amount of vitellogenic resorption and developed ~12% more eggs. Taken together, these results suggest that previtellogenic nutrition alters the extent and pattern of resorption after a blood meal through the effect of JH on mRNA abundance and lipid accumulation in previtellogenic follicles.

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1. Introduction

A broad examination of the various life history strategies among insects repeatedly demonstrates that reproductive output and nutritional status are coordinated to ensure survival and maximize long-term fitness. When nutrition is limited or environmental conditions are not ideal, reproductive trade-offs frequently manifest through the resorption of reproductive tissue (also described as: oosorption, follicular resorption or follicular atresia) (Reviewed in Bell and Bohm, 1975). Previous work has indicated that juvenile hormone (JH) is a key endocrine participant in the mediation of follicular resorption in response to nutritional limitation (Highnam et al., 1963a; Bell, 1971; Bell and Bohm, 1975; Tobe and Chapman, 1979; Weaver and Pratt, 1981; Clifton and Noriega, 2011). In insects that produce eggs continuously and sequentially, the relationship between JH, follicular resorption, and nutrition is usually direct and causal. Yolk protein production and oogenesis is often dependent on JH synthesis and overall nutritional status. When nutrition is limited, JH synthesis ceases, yolk protein synthesis is reduced and ovarian oosorption increases (Bell, 1971; Bell and Bohm, 1975; Tobe and Chapman, 1979; Weaver and Pratt, 1981). By coordinating the relationship between nutritional status and reproductive output through follicular resorption, JH participates directly in the reproductive decisions that inform an insect's life-history strategy (Reviewed in Flatt et al., 2005; Zera and Zhao, 2004; Harshman and Zera, 2007; Fronstin and Hatle, 2008). However, a conceptual framework that depends on a close linkage between JH, nutrition, and resorption cannot fully explain how fecundity and nutrition are coordinated in mosquitoes such as

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0022-1910/$ - see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.jinsphys.2012.05.005

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Aedes aegypti where oogenesis is discontinuous, dependent on a blood meal, and controlled by alternating titers of JH and ecdysone.

In anautogenic mosquitoes, reductions in fecundity through follicular resorption are known to occur during two main developmental stages: (1) a sugar-feeding previtellogenic stage governed primarily by JH (Gwadz and Spielman, 1973; Feinsod and Spielman, 1980; Raikhel and Lea, 1985; reviewed in Klowden, 1997) and (2) a blood-meal dependent vitellogenic stage governed primarily by ecdysteroids (Spielman et al., 1971; Fallon et al., 1974; Clements, 1992; reviewed in Klowden, 1997). After initial ovarian maturation is complete (60 h post eclosion), the mosquito enters a previtellogenic resting stage that lasts until a blood meal is found (Clements, 1992). During this period, JH synthetic rates slowly decline from their peak while ovarian follicles are resorbed through an apoptosis-like mechanism (Li et al., 2003; Clifton and Noriega, 2011). Resorption during this time is dependent on the quality of nutrition obtained through sugar feeding as well as JH. Sugar feeding and nutrition have important implications for fecundity during the previtellogenic resting stage as up to 20% of follicles can be resorbed in starved mosquitoes (Clifton and Noriega, 2011).

The first day after a blood meal in mosquitoes is marked by a rapid decrease in JH synthesis to <5 fmol/pair CA/hour, the lowest level seen during a gonotrophic cycle in *A. aegypti* (Li et al., 2003). By 20 h after a blood meal, ecdysteroid titers in the whole body of the mosquito have increased at least 5-fold from their original levels (Greenplate et al., 1985). In response to amino acids from the blood meal (Hansen et al., 2004) and high ecdysteroid titers (Martin et al., 2001) the fat body synthesizes vitellogenin and other yolk proteins (Raikhel and Lea, 1983; Cho et al., 1991, 1999; Sun et al., 2000) and mobilizes between ~50% and 80% of previtellogenic fat body lipid reserves for energy production and for incorporation into the oocytes (Briegel et al., 2002; Ziegler and Ibrahim, 2001; Zhou et al., 2004a). Significant nutritional partitioning of resources occurs after a blood meal as previtellogenic nutrition is not utilized in the same manner and cannot be considered interchangeable with blood meal derived nutrition during oogenesis (Zhou et al., 2004a,b). In *A. aegypti*, 19% of radiolabeled amino acids within the blood meal are deaminated and returned to the fat body to replace lipids transferred to the oocytes, while only 10% of the amino acids in a blood meal are directly incorporated into eggs (Zhou et al., 2004a,b). The substantial remaining portion of the blood meal is used in energy production (43%) or is excreted as waste (29%) (Zhou et al., 2004b). Since the nutritional content of eggs is substantially derived from previtellogenic nutritional reserves, it is not clear how reproductive output during vitellogenesis can be coordinated (via vitellogenic resorption) with these previtellogenic nutritional reserves in the absence of any significant JH synthesis.

After a blood meal, the oocytes and nurse cells show rapid increases in the synthesis of components required for the endocytosis of yolk proteins and lipids such as the: lipophorin receptor (Cheon et al., 2001; Seo et al., 2003; Sun et al., 2000), vitellogenin receptor (Sappington et al., 1996; Cho and Raikhel, 2001), and heavy-chain clathrin (Kokoza and Raikhel, 1997; Kokoza et al., 1997). The synthesis of endocytotic components in the follicles occurs in parallel with the synthesis and transfer of yolk components by the fat body (Roth and Porter, 1964; Koller and Raikhel, 1991; Reviewed in Raikhel and Dhadialla, 1992). Nearly all follicles that are remaining at the time of a blood meal will begin incorporating yolk proteins and lipids (Clements and Boocock, 1984). However, up to 27% of follicles will later become resorbed despite sometimes advanced vitellogenic development (Lea et al., 1978; Clements and Boocock, 1984). The mechanism or factors that allows one follicle to develop to maturity while another resorbs in the same ovary has not been described but has been suggested to involve "competition" between developing follicles for limited yolk components with the disadvantaged follicles eventually being resorbed (Highnam et al., 1963b; Schlaeger and Fuchs, 1974; Bell and Bohm, 1975) and their yolk components possibly returned to the pool of available nutrients (Bell, 1971). By 72 h post blood meal, a synchronous batch of eggs, adjusted over two developmental stages according to nutritional status, is ready for oviposition.

It is well documented that the final quantity of eggs produced (and as a corollary, the amount of vitellogenic resorption) is dependent in part on the quantity of blood ingested when comparing mosquitoes with equal previtellogenic nutritional reserves (Woke et al., 1956; Colless and Chellapah, 1960; Jalil 1974; Lea et al., 1978). Other work has shown that mosquitoes with low previtellogenic reserves will take substantially larger blood meals than high reserve mosquitoes but will still fail to develop an equal number of eggs. Conversely, mosquitoes with high reserves will take smaller blood meals but will always develop more eggs than low reserve mosquitoes (Nayar and Sauerman, 1975; Mostowy and Foster, 2004). Although a large blood meal can affect fecundity, it cannot prevent all vitellogenic resorption (Lea et al., 1978; Clements and Boocock, 1984) nor can it rescue the fecundity of mosquitoes with low nutritional reserves (Mostowy and Foster, 2004). These results, when taken together, suggest that most decisions about final reproductive output are made in response to nutrition during the previtellogenic resting stage and this information is integrated with blood feeding behavior to determine final fecundity as previously suggested in a conceptual model by Mostowy and Foster (2004). However, no hormonal or molecular mechanism has been elucidated which can explain how nutrition during the previtellogenic resting stage can affect blood feeding behavior or vitellogenic resorption.

Previous work in our laboratory has demonstrated that JH likely mediates follicular resorption in response to nutrition during the previtellogenic resting stage (Clifton and Noriega, 2011). During the course of that work it became apparent that nutritional and hormonal manipulations resulted in clear morphological changes to the previtellogenic ovary beyond only follicular resorption. These observations suggested that JH may mediate the relationship between fecundity and nutrition during the gonotrophic cycle of the mosquito in two ways: (1) through immediate previtellogenic resorption of follicles as previous work has shown (Clifton and Noriega, 2011) and (2) by altering intrinsic previtellogenic follicle "quality", physiology, and competitiveness during vitellogenesis thereby predetermining the fate of follicles after a blood meal. This hypothesis provides an endocrine mechanism whereby JH can mediate the full course of reproductive trade-offs in anautogenic mosquitoes during the previtellogenic and vitellogenic stages despite being nearly completely absent when many of these reproductive trade-offs are actually made (i.e. during vitellogenesis) (Li et al., 2003). To support this hypothesis, we explored three main questions: (1) Do changes in nutrition during the previtellogenic resting stage lead to relevant biochemical and molecular changes in the previtellogenic ovary? (2) Do hormonal manipulations during the previtellogenic resting stage lead to the same biochemical and molecular changes? (3) Does nutrition and hormones during the resting stage affect vitellogenic resorption and reproductive output? Together these questions demonstrate how the nutritional and hormonal conditions before a blood meal affect events after a blood meal and begin to explain one endocrine mechanism whereby previtellogenic nutrition (sugar feeding) is integrated with blood meal derived nutrition to determine fecundity.

2. Methods

2.1. Nutritional manipulations of insects

A colony of *A. aegypti* of the Rockefeller strain was maintained at 28 °C with 80% relative humidity under a 16 h day–8 h night

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regime. To control for any possible mating effects and ensure complete mating, males and females emerging during the same 15 h overnight period were collected and transferred to enclosures containing a ~2:1 excess of male mosquitoes. All mosquitoes were offered a cotton pad soaked in 3% sucrose solution for 2 days. On the third day post-eclosion, all mosquitoes were switched to one of three conditions: water only, 3% sucrose or 20% sucrose solution before being assayed.

2.2. Hormonal manipulations of insects

At 3 days post-emergence, randomly selected mosquitoes reared as previously described were cold anesthetized and topically treated with either 500 ng of methoprene (Zeocon Co., Palo Alto, CA) dissolved in 0.5 μL acetone or with 0.5 μL acetone alone. Mosquitoes were returned to a cotton pad soaked in 3% sucrose solution for 2 days until being dissected for qPCR or lipid quantification. Mosquitoes treated with JH III (Sigma–Aldrich, St. Louis, MO) were cold anesthetized at 3 days old and topically treated with 316 ng of JH III dissolved in 1 μL acetone or with 1 μL acetone alone. At 4 days old mosquitoes were treated a second time with JH III or acetone. Mosquitoes treated with JH III or acetone were blood fed on day 5 and the extent of resorption during oogenesis as well as final reproductive output was determined.

2.3. Ovarian triglyceride assay

Lipids were detected in the ovaries of mosquitoes maintained under the described experimental conditions using a triglyceride quantification kit (K622-100, Biovision, Mountain View, CA). In this kit, mono-, di- and triglycerides are hydrolyzed into glycerol and free fatty acids. Glycerol is then oxidized to generate a product which reacts with a colorimetric probe. The absorbance of the colorimetric probe can then be read at OD 570 nm. For each experimental treatment, 5 pairs of ovaries were dissected in *Aedes* physiological saline (APS) ($MgCl_2$ 0.6 mM; KCl 4.0 mM; $NaHCO_3$ 1.8 mM; NaCl 150.0 mM; HEPES 25.0 mM; $CaCl_2$ 1.7 mM). The ovaries were thoroughly cleaned of any contaminating fat body tissue, rinsed with PBS and placed in 100 μL of 5% nonidet P-40 detergent solution. After brief sonication, any remaining ovary lysate on the probe of the sonicator was rinsed back into the sample with 400 μL of ddH_2O. The ovarian lysate was then processed according to the kit instructions. Absorbance was read in a cuvette with a Nanodrop 2000c (Thermo Fisher Scientific, Waltham, MA). The lipase-negative glycerol control was used to blank the Nanodrop before each sample was read thereby subtracting background glycerol.

2.4. Ovarian micrographs and staining with oil red-O

Ovaries from mosquitoes were dissected in APS before being fixed in 4% paraformaldehyde for 1 h. The formaldehyde was replaced with 100 μL of oil red-O working solution for 1 h. Oil red-O specifically stains triglycerides and cholesteryl oleate and no other lipids (Ramirez-Zacarias et al., 1992). The ovaries were removed from the stain and rinsed thoroughly in PBS before being placed under a coverslip and photographed. Photographs were taken of the previtellogenic ovaries using a DM 5500 B Leica fluorescence microscope, a Leica DFC 310 FX mounted camera and Leica LAS imaging software.

2.5. Quantitative realtime qPCR

Total RNA was isolated from ovaries using RNA-binding glass powder as previously described (Noriega and Wells, 1993). Contaminating genomic DNA was removed using the DNA-*free*™ kit (Ambion, Austin, TX). Reverse transcription was carried out using the Verso cDNA Kit (Thermo Fisher Scientific Inc., Waltham, MA) by an oligo dT priming method according to the manufacturer's recommendations, using 300 ng of total RNA in 20 μL reactions. Real time qPCR was performed with the 7300 Real Time PCR System using TaqMan® Gene Expression Assays together with TaqMan® Universal PCR Master Mix (Applied Biosystems, Foster City, CA). The primer probes sequences for the 60S ribosomal protein L32 (rpL*32*, AAEL003396, VectorBase) Ovary-specific Lipophorin receptor (*Aa*LpR*ov*, AF355595, VectorBase) Heavy-chain clathrin (*Aa*CHC, AAEL013614-RA, VectorBase) and the vitellogenin receptor (*Aa*VgR, AAEL007657, VectorBase) genes are provided in Supplemental Table 1.

Primer/probes were synthesized by Applied Biosystems and reactions were done in a 20 μL volume according to manufacturer recommendations for Custom TaqMan® Gene Expression Assays. Reactions were run in triplicate using 1 μL of cDNA per reaction. Standard curves to quantify relative gene copy number were made from serial dilutions of plasmids containing rpL32, *Aa*VgR, *Aa*LpR*ov*, and *AaCHC* (300,000, 30,000, 3000, 300 and 30 copies of a plasmid per reaction). Real time data were collected by 7300 System SDS Software and analyzed in Microsoft Excel. Because many aspects of ovary and follicle physiology are heavily dependent on nutrition and hormones, traditional methods of normalization (i.e. normalizing to ribosomal, actin, or other housekeeping genes) biases the expression changes observed in previtellogenic oocytes (reviewed in Wrenzycki et al., 2007). Therefore, an alternative approach to normalizing mRNA abundance is required when working with oocytes (Wrenzycki et al., 2007). All genes were normalized to the average total follicle count of mosquitoes collected in triplicate of the same age and treatment to represent gene copy per average follicle.

2.6. Determination of blood meal mass

Mosquitoes from a common cohort were reared under the nutritional conditions previously described until 5 days old and divided into two groups. Female mosquitoes in the blood fed group were starved for 4–5 h before being offered porcine blood equilibrated to 37 °C with ATP added to a final concentration of 1 mM immediately before use. After feeding, mosquitoes from both the fed and unfed group were dried in an incubator at 65 °C for 5 days before measurement. The dry mass of the blood meal was calculated as the average dry mass of mosquitoes before a blood meal subtracted from the average dry mass of mosquitoes after a blood meal. At least 20 mosquitoes per treatment were dried and weighed.

2.7. Observations of ovarian resorption, final egg output and viability

Mosquitoes within each feeding and hormonal regime were blood fed as previously described. Mosquitoes from at least two independent replicates were anesthetized by chilling for 5–10 min at 4 °C prior to dissection. At 24 h after a blood meal, ovaries were dissected, rinsed in APS and stained with 0.5% neutral red solution in acetate buffer at pH 5.2 (Sigma–Aldrich, St. Louis, MO) for 10s to visualize resorbing follicles. Neutral red stains the lysosomes associated with resorbing follicles and can clearly indicate follicle status (Winckler, 1974; Bell and Bohm, 1975; Clements and Boocock, 1984; Hopwood et al., 2001). The ovaries were rinsed a second time in APS and placed under a coverslip. Photographs were taken as described in Section 2.4. Ovaries were later scored using Leica LAS imaging software for total follicle count and also for the presence of any resorbing follicles. To determine total egg output, mosquitoes fed 20% sucrose and 3% sucrose as well as mosquitoes treated with acetone and JH III were blood fed in triplicate

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as previously described and allowed to lay eggs on paper. The total number of eggs was counted by dissecting microscope and divided by the number of females observed to have blood fed to determine average number of eggs per female. Twelve days after oviposition, eggs from all treatments were hatched and the total number of unhatched eggs was counted to determine egg viability.

3. Results

3.1. Ovary morphology reflects overall nutritional reserves

The effect of nutrition on previtellogenic ovaries was explored my maintaining mosquitoes under three nutritional regimes: (1) water alone, (2) 3% sucrose in water, and (3) 20% sucrose in water. Ovaries from mosquitoes reared under such conditions show differences in follicle size, oocyte size, oocyte composition (as indicated by oil red-O staining of neutral lipids), and overall morphology (Fig. 1A). Ovarian morphology appears to be reflective of the overall nutritional reserves of mosquitoes as indicated by the dry mass of mosquitoes kept under varying nutritional regimes (Fig. 1B). Dry mass can be a suitable proxy for indicating nutritional reserves (Mostowy and Foster, 2004). Mosquitoes kept under increasing sucrose concentrations show statistically significant increases in dry body mass for each concentration offered (unpaired *t*-test; $p < .001$). Mosquitoes offered 20% sucrose accumulated nearly double the dry mass of those mosquitoes offered water alone (Fig. 1B).

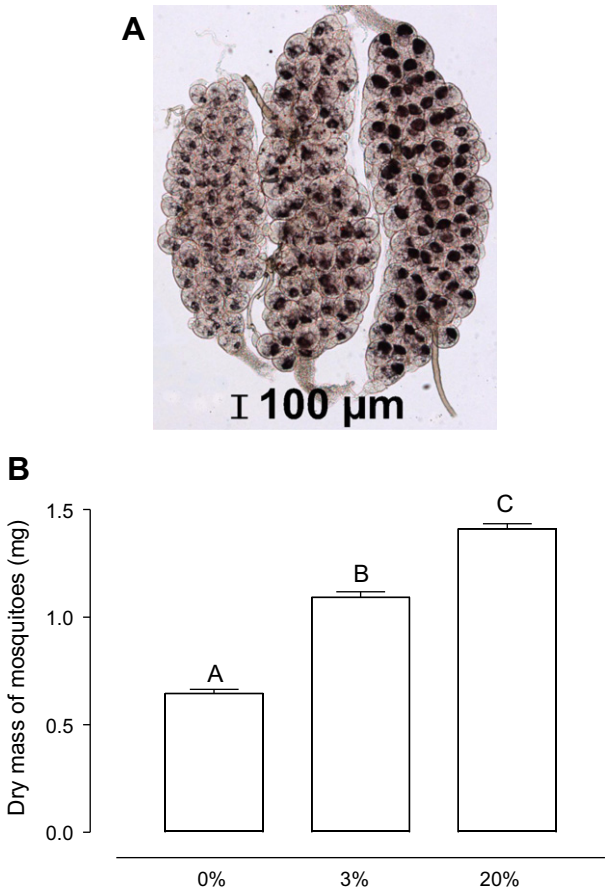

Fig. 1. Previtellogenic ovary morphology reflects previtellogenic nutritional reserves. (A) Representative images of single ovaries from mosquitoes maintained with water alone, 3% sucrose solution, and 20% sucrose solution. (B) Dry mass of mosquitoes maintained with water alone (0%), 3% sucrose (3%), and 20% sucrose (20%) (Mean ± SEM of ⩾20 mosquitoes per treatment). The dry mass of mosquitoes significantly increases as the concentration of sucrose offered increases (unpaired *t*-test; treatments with different letters denote statistical significance at $p \leqslant .001$).

3.2. Previtellogenic nutrition alters neutral lipid content of ovaries

To investigate the apparent differences in ovarian and follicle morphology more thoroughly, a neutral lipid specific stain (oil red-O) was used to stain the ovaries and visualize the accumulation of lipids in the oocytes. Ovaries from mosquitoes fed 20% sucrose concentrations show increased lipid accumulation as compared to those fed water or 3% sucrose (Fig. 2A). The densest accumulation of neutral lipids appears to be in the oocyte followed by the proximal nurse cells (Fig. 2A). The nurse cells furthest from the oocyte show less association with accumulated lipids. Accumulations of lipids within the oocyte appear to be most pronounced in mosquitoes fed the highest sucrose concentrations (Fig. 2A).

Since the content of lipids in the ovary appeared to change depending on previtellogenic nutrition, ovarian lipids may be an important energy source for transcriptional, translational and endocytotic activities during vitellogenesis. To begin exploring this possibility, the neutral lipid content of ovaries was quantified through the modification of a blood serum triglyceride quantification kit. Ovaries from mosquitoes fed 20% sucrose contained the highest content of stored neutral lipids at 581 pmol./female (Fig. 2B). This two-fold increase in ovarian lipids is significantly higher than mosquitoes fed either 3% sucrose or water alone (Fig. 2B; $p < .0001$; Mann–Whitney test). Feeding 3% sucrose did not significantly increase stored ovarian lipids over those fed water alone (Fig. 2B).

3.3. Previtellogenic nutrition alters expression of genes important for vitellogenesis

In conjunction with an examination of the energetic capacity of the follicles (i.e. stored lipids) we hypothesized that the expression of other cellular components important during vitellogenesis would be dependent on previtellogenic nutritional status. We chose to examine the expression of a ribosomal protein (60s subunit, rpL32) required for translation and three additional components required for the massive endocytosis associated with vitellogenesis but also expressed in lower levels during the previtellogenic resting stage: (1) The vitellogenin receptor (*Aa*VgR), (2) the ovary-specific isoform of the lipophorin receptor (*Aa*LpRov), and (3) an ovary-specific form of Heavy-Chain Clathrin (*Aa*CHC). For almost every gene and treatment, the number of transcripts per follicle increased as sucrose concentration increased (Fig. 3A–D). These differences were always significant when female mosquitoes fed water alone and those fed 20% sucrose were compared (Fig. 3A–D).

3.4. Previtellogenic methoprene application increases stored ovarian lipids

Previous work indicated that JH during the resting stage was an important regulator of fecundity by preventing follicular resorption (Clifton and Noriega, 2011). To investigate a possible role for JH in regulating the biochemical and molecular characteristics of the ovary during the resting stage, hormonal manipulations with methoprene were performed. Methoprene application alters the morphology of the follicle similar to the effect seen with 20% sucrose (Figs. 2 and 4A). Oocytes of mosquitoes treated with methoprene appear to contain massive increases in lipid content over acetone controls after staining with oil red-O (Fig. 4A). Quantification of ovarian lipids revealed that methoprene application causes a 8-fold increase in lipid content over acetone treatment alone in mosquitoes fed 3% sucrose (2760 pmol. vs. 333 pmol.

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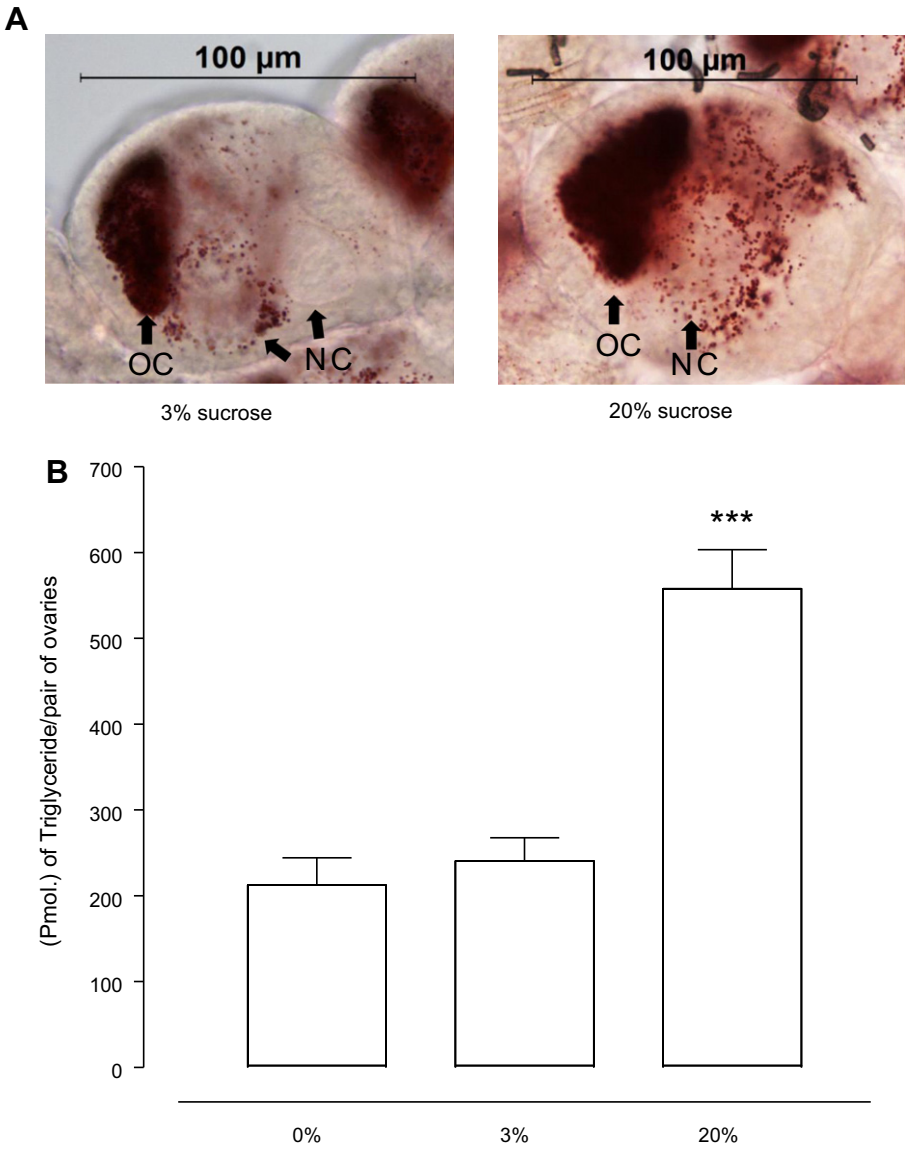

Fig. 2. Lipid accumulation in the follicle increases in mosquitoes offered 20% sucrose. (A) Representative images of a single follicle from mosquitoes maintained with 3% sucrose and 20% sucrose. Lipids accumulate predominantly in the oocyte (OC) and proximal nurse cells (NC) as indicated by oil red-O staining of neutral lipids. (B) The quantity of mono- di- and triglycerides in the ovaries significantly increases when mosquitoes are fed 20% sucrose as compared to those fed water alone (0%) or 3% sucrose solution (3%). (Mean ± SEM of at least 3 groups of 5 ovaries per treatment collected for at least three independent experiments; Mann–Whitney *U*-test; $^{***}p \leqslant .001$).

respectively; Fig. 4B; Mann–Whitney test; $p < .01$). In mosquitoes fed 20% sucrose, methoprene treatment causes a similar increase in the lipid content of the oocyte as compared to acetone controls (4180 vs. 702 pmol.; Fig. 4B; Mann–Whitney test; $p \leqslant .05$). Mosquitoes fed water alone and treated with methoprene also show a significant increase in the lipid content of oocytes when compared to acetone controls (1906 vs. 257 pmol.; Fig. 4B; Mann–Whitney test; $p \leqslant .05$).

3.5. Previtellogenic methoprene application alters expression of genes important for vitellogenesis

A comparison of the effect of methoprene on the expression of genes important to translation and endocytosis during vitellogenesis (rpL32, *Aa*VgR, *Aa*LpRov, *Aa*CHC) reveals that the effect of methoprene is similar to that of nutrition (Fig. 5A–D). Ribosomal protein L32of the 60s subunit showed a significant 38% increase in expression over controls with methoprene treatment (Fig. 5A). *Aa*LpRov also increased significantly 76% compared to acetone controls (Fig. 5B). *Aa*CHC increased significantly 51% compared to acetone controls (Fig. 5D) and although not statistically significant due to the high variability in previtellogenic expression observed with this receptor, *Aa*VgR increased 61% on average (Fig. 5C; $p = .09$; unpaired *t*-test). In each case, the effect of methoprene mimicked the effect of a 20% sucrose feeding regime.

3.6. The fate of follicles after a blood meal is affected by previtellogenic nutrition

To discount the possibility that reproductive decisions made during the resting stage can be rescued by a blood meal and confirm that previtellogenic nutrition (and its associated effects on gene expression and ovarian lipid content) affects the rate of resorption after a blood meal, the dry mass of a blood meal was determined for mosquitoes fed water alone, 3% sucrose and 20% sucrose (Fig. 6). As sugar concentration increased, (and nutritional

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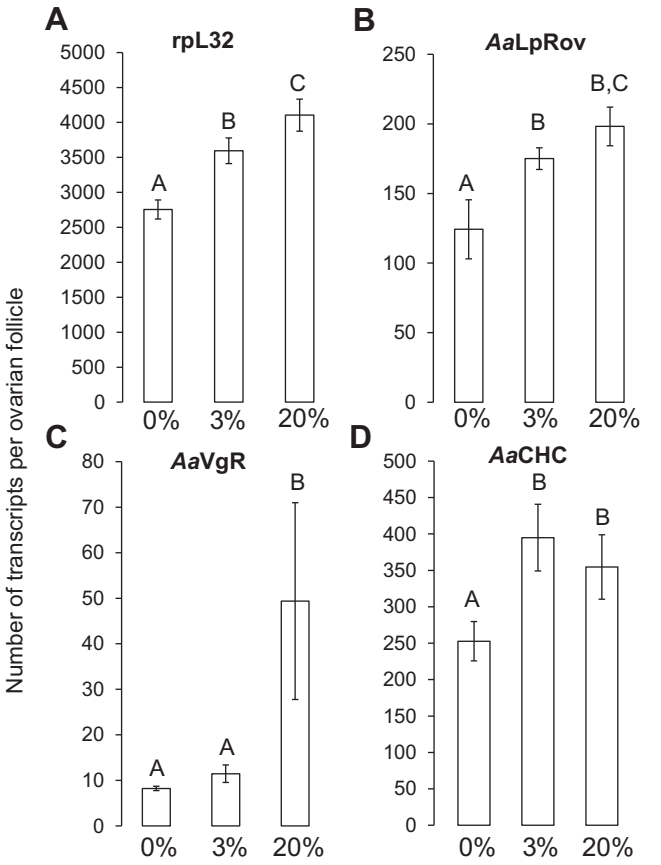

Fig. 3. Abundance of mRNA transcripts for (A) ribosomal 60s protein rpL32, (B) lipophorin receptor AaLpRov, (C) vitellogenin receptor (AaVgR) and (D) Heavy-Chain Clathrin AaCHC in the ovaries of mosquitoes fed water only (0%), 3% sucrose solution (3%), or 20% sucrose solution (20%). (Mean ± SEM of 5 pairs of ovaries collected for 4 replicates per treatment; unpaired *t*-test; treatments with different letters denote statistical significance at $p \leqslant .05$).

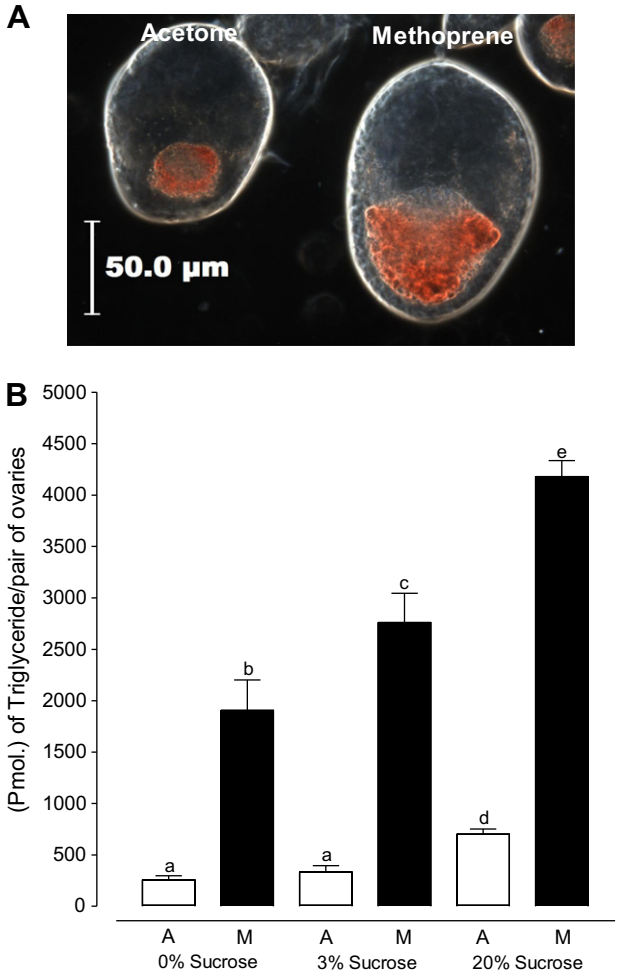

Fig. 4. Lipid accumulation in the follicle increases when mosquitoes are treated with methoprene. (A) Representative images of a single follicle from mosquitoes maintained with 3% sucrose and treated with methoprene dissolved in acetone or acetone alone. Lipids accumulate predominantly in the oocyte and proximal nurse cells as indicated by oil red-O staining of neutral lipids. (B) The quantity of mono-di- and triglycerides in the ovaries increases dramatically when mosquitoes fed water alone (0%), 3% or 20% sucrose are treated with methoprene (Mean ± SEM of 5 pairs of ovaries collected for at least 4 replicates per treatment; Mann–Whitney *U*-test; different letters denote significance $p \leqslant .05$).

reserves increased as shown by Fig. 1B) the mass of the blood meal decreased (Fig. 6). Mosquitoes that were maintained on 20% sucrose had a blood meal mass that was nearly 70% less than mosquitoes fed water alone (Fig. 6). The decrease in dry blood meal mass was statistically significant for each treatment ($p < .001$; unpaired *t*-test).

To confirm the effect previtellogenic nutrition and hormones have on the fate of follicles after a blood meal, mosquitoes were maintained on 3% sucrose and 20% sucrose concentrations and were fed blood. Ovaries from mosquitoes maintained on 3% sucrose and then examined 24 h after a blood meal showed a significantly higher rate of follicular resorption (Fig. 7A; $p < .001$; unpaired *t*-test) and those follicles that were not resorbing were smaller on average than follicles from mosquitoes fed 20% sucrose (197.8 µm vs. 203.2 µm; $p < .001$; unpaired *t*-test). Mosquitoes that were fed 20% sucrose before a blood meal had nearly three times less resorption than those fed 3% sucrose (13.6% vs. 4.8%; Fig. 7A). Mosquitoes fed 20% sucrose also laid on average 10.4 more eggs per female (117.3 vs. 106.9; $p < .05$; unpaired *t*-test; Fig. 7B). The effect of previtellogenic JH III treatment on vitellogenic resorption is similar to the effect of offering high sucrose concentrations. Mosquitoes fed 3% sucrose and treated with JH III had 40% less resorption 24 h after a blood meal (12.6% vs. 7.6%; Fig. 7C). Mosquitoes treated with JH III also laid on average 11.2 more eggs per female than those mosquitoes treated with acetone alone (104.4 vs. 93.2; $p < .01$; unpaired *t*-test; Fig. 7D). However, the proportion of eggs laid that successfully hatched was not significantly altered by either JH III treatment or feeding 20% sucrose over controls (results not shown).

4. Discussion

4.1. Defining oocyte and follicle quality

Central to any hypothesis that relies on the differential survival of oocytes during oogenesis is the idea that oocytes vary in their inherent ability to develop and so exist as part of a heterogeneous population. Therefore, the "quality" of an oocyte is likely to determine its potential for further development (reviewed in Gilchrist et al., 2008). While oocyte quality may mean different things depending on the observational context as well as the specific developmental features of an organism, Thomson et al., 2010, define oocyte quality broadly as "...[the] intrinsic genetic, epigenetic and cytoplasmic characteristics required for the completion of development and capability of producing a normal offspring". We examined likely indicators of a follicles ability to complete vitellogenic development (i.e. "quality") such as: oocyte neutral lipid content, lipophorin receptor expression (*Aa*LpRov) (Cheon et al.,

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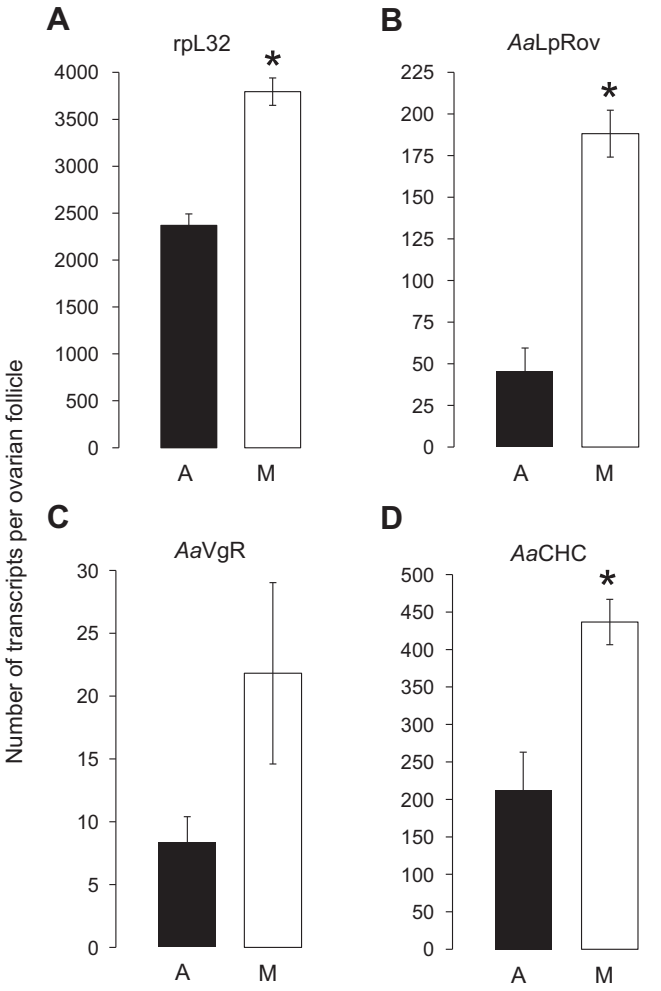

Fig. 5. Abundance of mRNA transcripts for (A) ribosomal 60s protein rpL32, (B) lipophorin receptor (*Aa*LpRov), (C) vitellogenin receptor, (*Aa*VgR) and (D) Heavy-Chain Clathrin (*Aa*CHC) in the ovaries of mosquitoes treated with methoprene (M = methoprene treatment) or with acetone (A = acetone treatment). (Mean ± SEM of 5 pairs of ovaries collected for 4 replicates per treatment; unpaired *t*-test; $^{*}p \leqslant .05$).

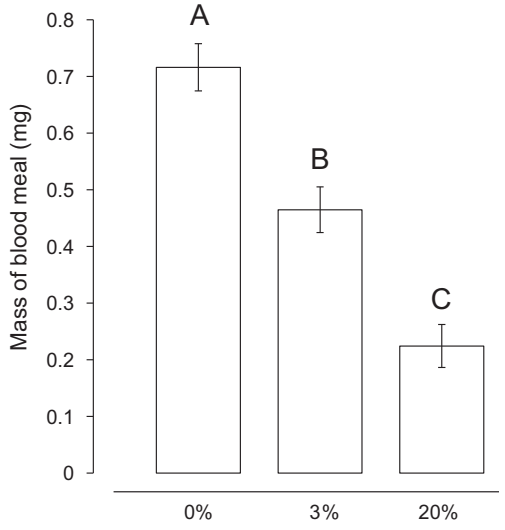

Fig. 6. Mosquitoes offered higher concentrations of sucrose take significantly smaller blood meals. (Mean ± SEM; unpaired *t*-test; treatments with different letters denote statistical significance at $p \leqslant .001$).

2001; Seo et al., 2003; Sun et al., 2000), vitellogenin receptor expression (*Aa*VgR) (Sappington et al., 1996; Cho and Raikhel, 2001), heavy-chain clathrin expression (*Aa*CHC) (Kokoza and Raikhel, 1997; Kokoza et al., 1997) as well as 60s ribosomal protein expression subunit L32 (rpL32). These markers of mosquito oocyte quality, while certainly not meant to be an exhaustive inventory, begin to illustrate how previtellogenic nutrition and JH synthesis can affect vitellogenic resorption by altering an oocyte's "...intrinsic developmental potential" (Gilchrist et al., 2008). Each of these indicators of follicle quality was altered in response to nearly all of the nutritional and hormonal manipulations conducted on previtellogenic mosquitoes. Furthermore, these same nutritional and hormonal manipulations clearly altered the rate of resorption after a blood meal as well as final egg output (Fig. 7A–D). The biochemical and transcriptional changes that occur in response to manipulations of previtellogenic nutritional and hormonal status (Figs. 2–5) strongly suggests that the rate of resorption after a blood meal is dependent on the quality of follicles beginning vitellogenesis and also that the quality of follicles is mediated through JH in response to nutrition. These results also suggest that the intrinsic development potential of a follicle (i.e. follicle quality) can be defined in *A. aegypti* by examining aspects of oocyte function that are likely to be important during vitellogenesis.

4.2. Oocyte lipids can be altered by previtellogenic nutrition as well as hormonal manipulation

Previtellogenic oocyte lipids may play an important and overlooked role in the reproductive physiology of *A. aegypti.* To our knowledge, only a few studies have been conducted that include any information about previtellogenic ovarian lipids. Tadkowski and Jones (1978) suggested that all lipid yolk contained within the oocyte is synthesized within the oocyte both before and after a blood meal. Troy et al. (1975) concluded that the principal previtellogenic ovarian lipids are hydrocarbons and fatty esters. However, both of these results were challenged in later work by Ziegler (1997) who found that while the ovary can synthesize and store triglycerides and phospholipids, they make up only 1/1000th of the final lipid content of mature eggs under *in vitro* culturing conditions. Ziegler and Ibrahim, 2001 later described the amount of lipid stored in previtellogenic ovaries as a "trace". To our knowledge, all previous studies have been specifically focused on the origins of the microgram quantities of lipids transferred to eggs after a blood meal and do not describe any potential role for previtellogenic oocyte lipids. The small quantity of previtellogenic triglycerides we examined in the follicles of the ovary are possibly the "minor" or "trace" proportion capable of being synthesized by the ovary itself as described by Ziegler and Ibrahim, 2001. However, viewing previtellogenic oocyte-derived lipids as an interchangeable proportion of final egg lipid reserves is probably incorrect and overly simplistic.

The presence of stored lipids in oocytes is widespread among animals, and has been correlated with oocyte "quality" and reproductive success (Nagano et al., 2006a,b; Angel-Dapa et al., 2010). The exact role of stored previtellogenic lipids in mosquito oocytes is unknown but current evidence suggests that oocyte lipids, at least in those vertebrate animals which have been studied, are an important substrate for energy production in oocytes (Sturmey and Leese, 2003; Ferguson and Leese, 2006; Reviewed in Sturmey et al., 2009). Furthermore, the composition of lipids synthesized or stored by oocytes is generally unique and enriched in specific saturated fatty acids and likely reflects the "... highly specialized requirements" of an oocyte (Homa et al., 1986; Matorras et al., 1998). Similar to vertebrate organisms, Zeigler's work with ovarian lipid synthesis in *A. aegypti* shows that lipids synthesized in the mosquito ovary are also specifically enriched in saturated fatty acids (1997). While

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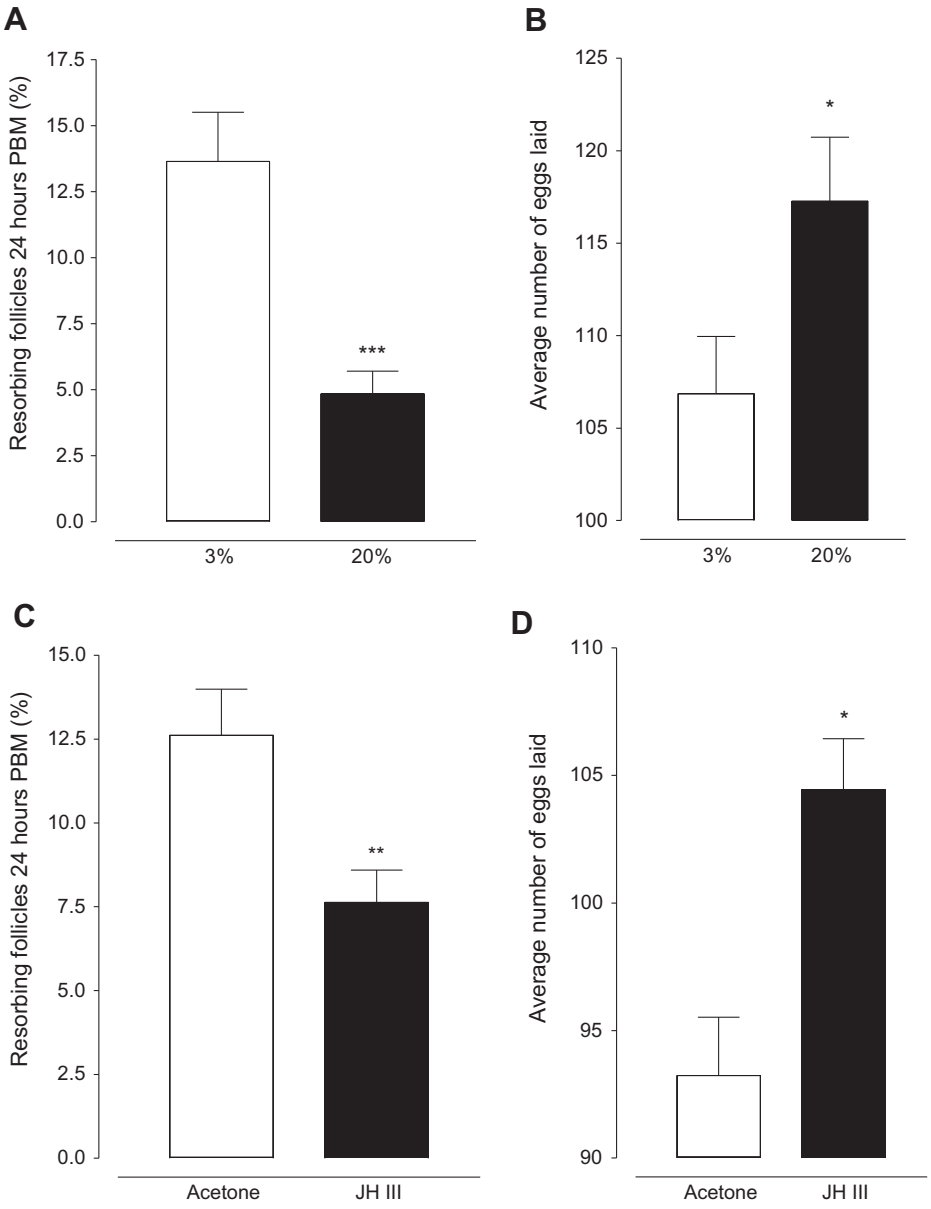

Fig. 7. Sugar feeding or hormone treatment during the previtellogenic resting stage alters the rate of resorption 24 h after a blood meal as well as total egg output. (A) Mosquitoes offered 20% sucrose show nearly 3-fold less resorption than mosquitoes offered 3% sucrose prior to a blood meal (Mean ± SEM of 10 pairs of ovaries collected in two independent replicates per treatment; unpaired *t*-test; $^{***}p \leqslant .001$). (B) Mosquitoes offered 20% sucrose laid 10.4 more eggs than mosquitoes fed 3% sucrose (Mean ± SEM of eggs from 21 females unpaired *t*-test; $^{*}p \leqslant .05$). (C) JH III application prior to a blood meal reduces vitellogenic resorption by 40% (Mean ± SEM of 15 pairs of ovaries collected in two independent replicates per treatment; unpaired *t*-test; $^{**}p \leqslant .01$). (D) Mosquitoes fed 3% sucrose and treated with JH III laid 11.2 more eggs than mosquitoes treated with acetone (Mean ± SEM of eggs from 21 females; unpaired *t*-test; $^{*}p \leqslant .05$).

the exact purpose of stored oocyte lipids and fatty acid enrichment is unclear, the work presented here in conjunction with existing literature does suggest that previtellogenic lipids synthesized and stored in the oocyte have a unique reproductive function which may not be the same as lipids derived from the fat body, which do not show saturated fatty acid enrichment (Ziegler (1997)).

The period after a blood meal in mosquitoes is marked by intense vitellogenin receptor, lipophorin receptor and heavy-chain clathrin synthesis by the oocyte and nurse cells (Sappington et al., 1996; Kokoza and Raikhel, 1997; Kokoza et al., 1997; Sun et al., 2000; Cheon et al., 2001; Cho and Raikhel, 2001; Seo et al., 2003), as well as massive endocytosis of yolk components (Reviewed in Raikhel and Dhadialla, 1992). Since endocytosis and protein synthesis are both energy-dependent processes, it is not unreasonable to suggest that a pool of previtellogenic oocyte lipids supports the energetic demands of the growing oocyte during at least the beginning stages of vitellogenesis. This pool of oocyte lipids is clearly affected by methoprene (Fig. 4A and B) as well as nutritional status (Fig. 2A and B) during the resting stage and may provide the energetic basis for an endocytotic "competition" between follicles for yolk components during vitellogenesis as suggested by Bell and Bohm (1975) as well as provide the basis for understanding why one follicle resorbs besides another which is developing (Lea et al., 1978; Clements and Boocock, 1984). While we did not specifically investigate the composition of ovarian lipids, the total quantity of neutral lipids stored in the ovary seems to reflect overall nutritional reserves (Fig. 2A and B). This result suggests that the pool of ovary lipids is dynamically regulated and integral to the coordination of fecundity with previtellogenic nutrition during vitellogenesis.

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4.3. Previtellogenic expression of the endocytotic complex can be altered by nutrition and methoprene application

We also examined the previtellogenic mRNA levels of three proteins integral to the rapid receptor-mediated endocytosis that occurs during vitellogenesis. Much previous work has demonstrated that the post-eclosion formation of an endocytotic complex is dependent on a peak of JH synthesis (Raikhel and Lea, 1985) and that the endocytotic complex is composed of vitellogenin receptors (*Aa*VgR) (Sappington et al., 1996; Cho and Raikhel, 2001), lipophorin receptors (*Aa*LpRov), (Cheon et al., 2001; Seo et al., 2003; Sun et al., 2000), as well as heavy-chain clathrin (*Aa*CHC) (Kokoza and Raikhel, 1997; Kokoza et al., 1997). Since the initial formation of an endocytotic complex in mosquito follicles is dependent on JH, and stored ovarian lipids were clearly altered during the previtellogenic resting stage in response to methoprene (Fig. 4A and B), we hypothesized that the transcription and accumulation of mRNA's for these genes would also be similarly modulated in response to hormones and nutrition. As our results suggest, JH not only induces the formation of an endocytotic complex, but may also maintain the expression of the components of the endocytotic complex in response to previtellogenic nutritional status.

Although limited evidence exists, Cho and Raikhel (2001) have suggested that transcripts of genes important to vitellogenesis are made by the nurse cells, transferred to the oocytes, and tend to accumulate during the previtellogenic resting stage. *In situ* hybridizations performed in previous studies for each of these endocytotic genes demonstrates substantial mRNA accumulation in the cytoplasm of the oocyte (Kokoza and Raikhel, 1997; Cho and Raikhel, 2001; Cheon et al., 2001; Seo et al., 2003). Presumably, the large-scale translation of these components to support endocytosis and vitellogenesis would then depend on a blood meal (stored oocyte lipids during this time would be especially useful in providing the energy for rapid up-regulation of the endocytotic complex) (Kokoza and Raikhel, 1997). Studies in vertebrate and invertebrate animals have indicated that in addition to neutral lipids, storage of mRNA and protein components necessary for development by the oocyte is a ubiquitous and vitally important determinant of oocyte quality that is often correlated with an oocyte's likelihood of successful fertilization and development (Brandhorst, 1980; Reviewed in Eichenlaub-Ritter and Peschke, 2002; Leoni et al., 2007; reviewed in Wrenzycki et al., 2007; reviewed in Mermillod et al., 2008).

In addition to endocytotic components, we also examined the abundance of mRNA's for the protein L32 of the ribosomal 60s subunit. In each treatment, the abundance of rpL32 mRNA behaved similarly to genes necessary for vitellogenesis. The abundance of ribosomal mRNA's in the ovary is likely related to the developmental capacity of the oocyte and reflects the capacity of the oocyte to synthesize proteins after a blood meal or during embryogenesis. In those animals which have been studied, the majority of ribosomal proteins (Bachvarova and De Leon, 1977) and ribosomal mRNA's (Burkholder et al., 1971; Reviewed in Eichenlaub-Ritter and Peschke, 2002) are stored in the oocyte for later use. While we did not study the function of the accumulations of ribosomal mRNA's it seems likely that the translational capacity of the oocyte would be co-regulated to match available energy (oocyte lipid stores) and available template material (mRNA's related to vitellogenesis).

In this study we attempted only to quantify the abundance of mRNA's for genes likely to be important after a blood meal in order to define and characterize the morphological changes we observed in previtellogenic ovaries (Fig. 1A). Because we only examined mRNA abundance on a per follicle basis we cannot be certain how translational events before or after a blood meal affect the expression of these endocytotic components. Despite this, the dependence of neutral lipids (Figs. 2 and 4) and endocytotic mRNA's (Figs. 3 and 5) on previtellogenic nutrition and hormones does suggest that a relationship between the pattern of accumulation of endocytotic mRNA's and a follicle's fate does exist. While we did not specifically explore the individual differences between follicles of the same ovary, it is likely that differences in mRNA accumulation do exist between follicles of the same ovary which would provide the basis for a vitellogenic "competition" for yolk components.

4.4. Low reserve mosquitoes take more blood but develop fewer eggs

A major factor that must be included in any explanation of vitellogenic resorption in mosquitoes is the role of a blood meal. Sugar feeding has been shown to affect blood meal size such that recently sugar fed mosquitoes with full crops can experience reductions in fecundity due to blood meal volume limitations in the abdomen (Mostowy and Foster, 2004). Additionally, a proportion of any reduction in fecundity can be attributed to previtellogenic resorption and is therefore completely independent of a blood meal (Supplemental Fig. 1; Clifton and Noriega, 2011). Despite crop-volume effects and previtellogenic resorption, the relationship between fecundity, blood meal size and previtellogenic nutrition still appears to be counterintuitive in *A. aegypti*, primarily because large blood meals cannot rescue low reserve mosquitoes even when crop volume is controlled (Mostowy and Foster, 2004).

To explain this incongruence, we would like to suggest that stored previtellogenic fat body reserves are not only important for direct incorporation into eggs, but may also be critically important in providing the energy to digest a blood meal and manufacture yolk proteins. The digestion of blood likely requires significant energy as evidenced by the increased rate of respiration after a blood meal (Gray and Bradley, 2003; Sarfati et al., 2005). Work by Zhou et al. (2004a) showed that ~29% of the blood meal is excreted as waste and ~43% is used as energy suggesting that there is some limit to the amount of nutrition that can actually be recovered from a blood meal for use in eggs. The same work has also shown that up to ~50% of mobilized fat body lipid reserves are utilized as energy after a blood meal (Zhou et al., 2004a). Therefore, the extent of previtellogenic nutritional reserves may partially dictate how much of the nutrition contained within a blood meal is ultimately recoverable and convertible into yolk components. The ubiquity of resorption after a blood meal in mosquitoes of all nutritional conditions strongly suggests that blood can never prevent all resorption and that yolk components are probably always in short supply during vitellogenesis (Lea et al., 1978; Clements and Boocock, 1984; Nayar and Sauerman, 1975; Mostowy and Foster, 2004). Taken together with the multitude of studies that have shown a clear relationship between the magnitude of resorption and blood meal size (Woke et al., 1956; Colless and Chellapah, 1960; Jalil, 1974; Lea et al., 1978; Mostowy and Foster, 2004), a limit to the nutrition that can be derived from a blood meal would explain the high levels of vitellogenic resorption seen in low reserve mosquitoes despite the acquisition of a large blood meal. In this context, previtellogenic lipid reserves may be doubly important by: (1) determining the extent of utilization of a blood meal and/or the extent of synthesis of yolk proteins and (2) for direct incorporation into follicles during oogenesis. Although we did not specifically explore how previtellogenic nutrition affects blood feeding behavior or blood meal utilization, it is clear that blood feeding behavior, previtellogenic nutritional status and fecundity are intricately related.

4.5. A model for understanding the coordination of reproductive output with nutrition in the anautogenic mosquito

The study presented here attempts to answer two lingering questions posed in the literature about the regulation of resorption and the reproductive physiology of anautogenic mosquitoes: (1)

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How does the mosquito determine the extent of resorption (i.e. coordinate fecundity) based on blood meal size and previtellogenic reserves? (Lea et al., 1978; Browne, 2001; Mostowy and Foster, 2004) and also (2) how does the mosquito decide which follicles to resorb? (Lea et al., 1978; Clements and Boocock, 1984). When the results of this study are integrated with existing literature, a regulatory scheme emerges that can potentially answer these questions and provide a conceptual framework with which to understand the coordination of reproductive output with nutrition in anautogenic mosquitoes (Fig. 8).

The work presented here suggests that JH during the resting stage regulates the physiology of a presumably heterogeneous

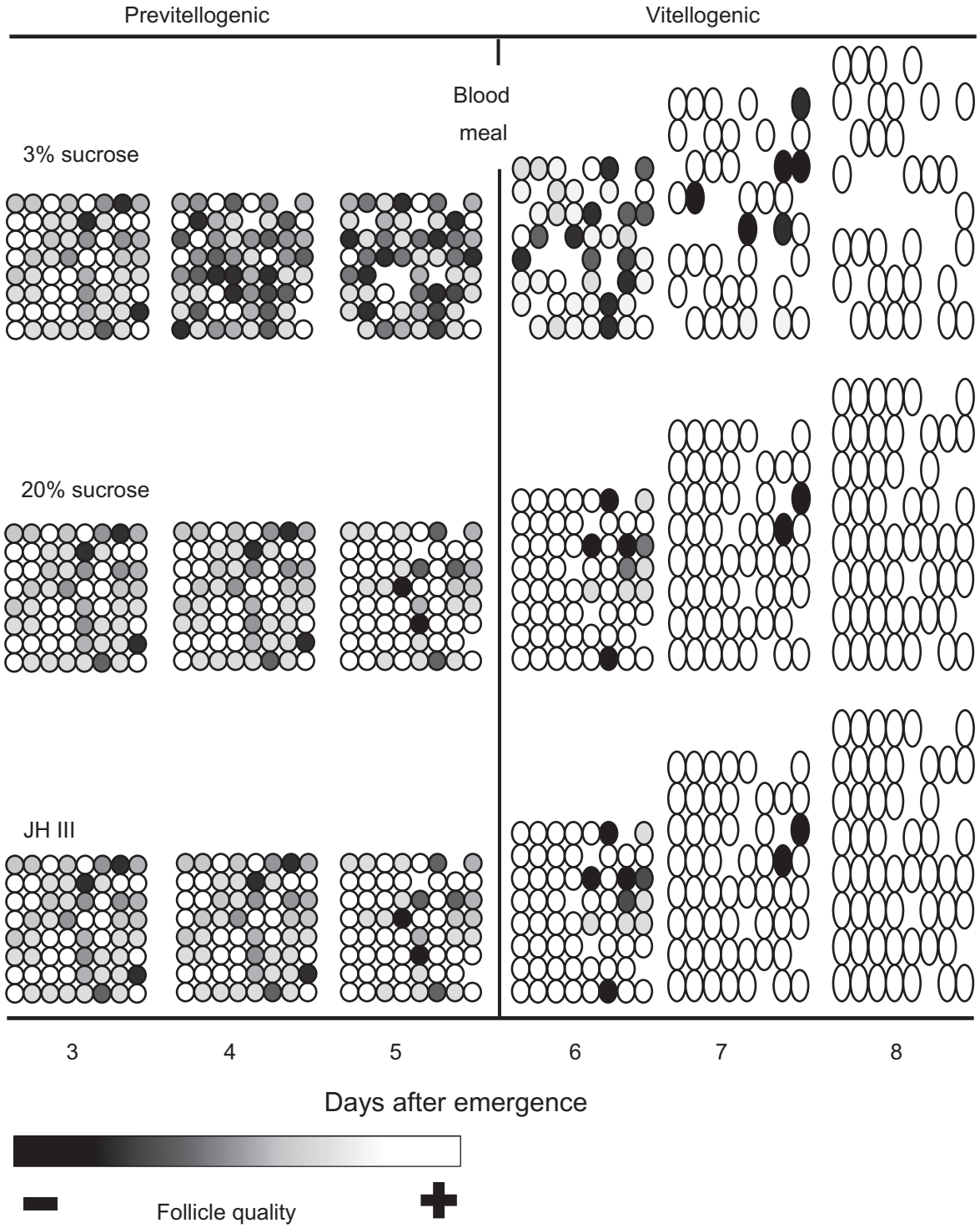

Fig. 8. A model that describes the effect of nutrition and hormones during the previtellogenic resting stage on follicular resorption after a blood meal. Extent of grey shading is indicative of follicle quality. Black follicles are being resorbed. Day 3: initial previtellogenic maturation of the ovarian follicles is completed and a heterogeneous population of follicles exists. Days 4 and 5: until a blood meal is found, follicles of lesser quality are resorbed and overall fecundity is reduced. Feeding 20% sucrose or applying JH III reduces resorption and increases average follicle quality. Day 6: after a blood meal, all remaining follicles begin vitellogenesis and a competition for yolk components. Day 7: as ecdysteroid titers increase and reach a peak, follicles of lower quality and lagging in vitellogenic development are resorbed in favor of those with advanced vitellogenic development. Day 8: a batch of eggs adjusted to match nutritional status during the previtellogenic resting stage and after a blood meal is ready for oviposition.

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population of previtellogenic follicles in response to previtellogenic nutrition. After a blood meal, all primary follicles begin depositing yolk components in the oocyte (Clements and Boocock, 1984; Lea et al., 1978). As a "competition" for these yolk components commences (as suggested by Bell and Bohm, 1975), those follicles which are lagging in development or otherwise unable to compete are ultimately resorbed in favor of those follicles which possess a better ability to develop (Fig. 8). The best support for such a competition comes from experiments by Schlaeger and Fuchs (1974) and confirmed in our laboratory in which high doses of 20-hydroxyecdysone injected into previtellogenic mosquitoes caused all follicles to begin depositing yolk, only later to be resorbed (~48 h) in favor of just a few highly developed follicles comprised solely of whatever endogenous nutritional reserves were available for incorporation at the time of ecdysteroid treatment (results not shown).

Although ecdysteroid injection induced massive resorption and the development of a few eggs, other work with *Caenorhabditis elegans* showed the consequences of preventing apoptosis and resorption in oocytes. When apoptosis was prevented, remaining oocytes showed smaller sizes and lower viability implying that the pool of reproductive resources was limited and dividing this pool among more oocytes had negative consequences. The authors concluded that a "competition" for resources among aging oocytes maintains oocyte quality by resorbing those of inferior quality (Andux and Ellis, 2008). In *Nauphoeta cinerea*, the same coordination between the pool of reproductive resources and egg number was shown when resources recovered from resorbing follicles were reinvested into the remaining follicles and were specifically not returned to the pool of somatic resources (Barrett et al., 2009). In *Periplaneta americana*, this phenomenon was taken a step further when yolk proteins from resorbing follicles were shown to be released into the haemolyph intact and therefore readily available to developing follicles (Bell, 1971). To our knowledge, Bell (1971) results have not been repeated in other insects. Although we did not specifically explore the fate of the resources recovered from resorbing follicles or the effects of blocking resorption, our results do suggest that during oogenesis a competition occurs between follicles, based in some part, on the intrinsic quality factors described here.

In addition to initiating further development, it is also likely that ecdysteroids provide the hormonal stimulus for resorption in developmentally inferior follicles (Fig. 8). Previous work has shown that an integral component of programmed cell death, the effector caspase *Ae*Dronc, is most highly expressed during pulses of ecdysteroids, especially during metamorphosis (Cooper et al., 2007). In the adult mosquito ovary, 20-hydroxyecdysone treatment causes a nearly 3-fold increase in *Ae*Dronc transcription (Cooper et al., 2007). In *Drosophila melanogaster*, ecdysteroid treatment clearly increases ovarian apoptosis and resorption (Soller et al., 1999; Terashima et al., 2005). Although ecdysteroid treatments induce apoptosis and resorption in insects with sequential egg development (thereby directly controlling reproductive output), it has been shown previously by Uchida et al. (1998) in *Culex pipiens pipiens* and reaffirmed for *A. aegypti* (Uchida and Moribayashi, 2002) that the ecdysteroid titer itself likely does not regulate the determination of egg number during vitellogenic development in insects with synchronous egg development (Browne, 2001). The failure of ecdysteroid titers to determine egg number suggests that a threshold concentration of ecdysteriods exists that induces resorption in sensitive follicles and that JH during previtellogenic development participates in determining the sensitivity of follicles to ecdysteriods. The reduction in resorption after a blood meal in those mosquitoes treated with JH III (Fig. 7A) during the resting stage further supports a role for JH during vitellogenic resorption.

A similar regulatory scheme has been described to explain follicular resorption in *D. melanogaster* by Terashima et al. (2005). In *D. melanogaster*, starvation causes a rapid increase in ecdysteroid titer followed by increased resorption of follicles (Terashima et al., 2005). Since some ecdysteroid titer is required in this species for normal oogenesis, an activating threshold can explain how ecdysteriods can be dually important for oogenesis as well as resorption (Terashima et al., 2005). The co-occurrence of vitellogenic resorption (Clements and Boocock, 1984) with high ecdysteroid titers (Greenplate et al., 1985) in *A. aegypti* also suggests that the onset of vitellogenic resorption in susceptible follicles is probably dictated by an ecdysteroid threshold after a blood meal. A regulatory scheme that relies on the differential sensitivity of follicles to a "threshold" of ecdysteroids would explain how one follicle resorbs beside another which is developing (Fig. 8). Despite the ability of JH and nutrition to alter the average quality of follicles, the exact mechanism that confers differential sensitivity to ecdysteriods is still unknown, although our results here suggest it is related to the intrinsic ability of the oocyte to incorporate yolk components and undergo vitellogenic development.

4.6. Conclusions

In this study we examined likely indicators of a follicles ability to complete vitellogenic development (i.e. "quality") such as: oocyte lipid content, lipophorin receptor expression, vitellogenin receptor expression, heavy chain clathrin expression and ribosomal protein expression. Each of the explored quality markers represents a small cross-section of the possible changes that occur in the oocyte in response to hormones and nutrition. However, a few key details about the regulatory scheme that the work here suggests remain unanswered. (1) How does previtellogenic nutritional status alter blood feeding behavior? (2) Does the extent of vitellogenic development within an individual follicle during the period of high ecdysteroid titer determine the likelihood of resorption? (3) How does the extent of vitellogenic development within a follicle determine the sensitivity of the follicle to ecdysteroids and the likelihood of resorption?

By modulating the follicles response to the hormonal and nutritional environment after a blood meal, JH seems to integrate previtellogenic nutritional information with blood meal derived nutrition to determine reproductive output. This regulatory scheme, by expanding a role for JH to include post-blood meal resorption, illustrates the endocrinological compromises that underlay the peculiarities of an anautogenic life history strategy such as a previtellogenic resting stage, sugar feeding, synchronous egg development, and hematophagy.

Acknowledgements

We thank Dr. Marcela Nouzova for rearing mosquitoes as well as Dr. Marcela Nouzova, Dr. Crisalejandra Rivera-Perez and Mario Perez for critical reading of the manuscript. This work was supported by NIH Grant No. AI 45545 to F.G.N.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jinsphys.2012.05.005.

References

Andux, S., Ellis, R.E., 2008. Apoptosis maintains oocyte quality in aging *Caenorhabditis elegans* females. Plos Genetics 4, 1–13.

Angel-Dapa, M.A., Rodríguez-Jaramillo, C., Cáceres-Martínez, C.J., Saucedo, P.E., 2010. Changes in lipid content of oocytes of the penshell *Atrina maura* as a criterion of gamete development and quality: A study of histochemistry and digital image analysis. Journal of Shellfish Research 29, 407–413.

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Bachvarova, R., De Leon, V., 1977. Stored and polysomal ribosomes of mouse ova. Developmental Biology 58, 248–254.

Barrett, E.B., Moore, A.J., Moore, P.J., 2009. A potential function for oocyte apoptosis in unmated *Nauphoeta cinerea*. Physiological Entomology 34, 272–277.

Bell, W.J., 1971. Starvation-induced oöcyte resorption and yolk protein salvage in *Periplaneta americana*. Journal of Insect Physiology 17, 1099–1111.

Burkholder, G.D., Comings, D.E., Okada, T.A., 1971. A storage form of ribosomes in mouse oocytes. Experimental Cell Research 69, 361–372.

Brandhorst, B.P., 1980. Simultaneous synthesis translation and storage of messenger RNA including histone messenger RNA in sea urchin *Lytechinus pictus* eggs. Developmental Biology 79, 139–148.

Briegel, H., Hefti, M., Di Marco, E., 2002. Lipid metabolism during sequential gonotrophic cycles in large and small females of *Aedes aegypti*. Journal of Insect Physiology 48, 547–554.

Browne, L.B. 2001. Quantitative aspects of the regulation of ovarian development in selected anautogenous Diptera: Integration of endocrinology and nutrition.

Bell, W.J., Bohm, M.K., 1975. Oosorption in insects. Biological Reviews 50, 373–396.

Cheon, H.M., Seo, S.J., Sun, J.X., Sappington, T.W., Raikhel, A.S., 2001. Molecular characterization of the VLDL receptor homolog mediating binding of lipophorin in oocyte of the mosquito *Aedes aegypti*. Insect Biochemistry and Molecular Biology 31, 753–760.

Cho, W., Tsao, S., Hays, A.R., Walter, R., Chen, J., Snigirevskaya, E., Raikhel, A.S., 1999. Mosquito cathepsin B-like protease involved in embryonic degradation of vitellin is produced as a latent extraovarian precursor. Journal of Biological Chemistry 274, 13311–13321.

Cho, W.L., Deitsch, K.W., Raikhel, A.S., 1991. An extraovarian protein accumulated in mosquito oocytes is a carboxypeptidase activated in embryos. Proceedings of the National Academies of Science 88, 10821–10824.

Cho, K.H., Raikhel, A.S., 2001. Organization and developmental expression of the mosquito vitellogenin receptor gene. Insect Molecular Biology and Biochemistry 10, 465–474.

Clements, A.N., 1992. The Biology of Mosquitoes. Chapman and Hall, London.

Clements, A.N., Boocock, M.R., 1984. Ovarian development in mosquitoes stages of growth and arrest and follicular resorption. Physiological Entomology 9, 1–8.

Clifton, M.E., Noriega, F.G., 2011. Nutrient limitation results in juvenile hormone-mediated resorption of previtellogenic ovarian follicles in mosquitoes. Journal of Insect Physiology 57, 1274–1281.

Colless, D.H., Chellapah, W.T., 1960. Effects of body weight and size of blood-meal upon egg production in *Aedes aegypti* (Linneaus) (Diptera: Culicidae). Annals of tropical medicine and parasitology 54, 475–482.

Cooper, D.M., Thi, E.P., Chamberlain, C.M., Pio, F., Lowenberger, C., 2007. Aedes Dronc: a novel ecdysone-inducible caspase in the yellow fever mosquito, *Aedes aegypti*. Insect Molecular Biology 16, 563–572.

Eichenlaub-Ritter, U., Peschke, M., 2002. Expression in in-vivo and in-vitro growing and maturing oocytes: focus on regulation of expression at the translational level. Human Reproduction Update 8, 21–41.

Fallon, A.M., Hagedorn, H.H., Wyatt, G.R., Laufer, H., 1974. Activation of vitellogenin synthesis in the mosquito *Aedes aegypti* by ecdysone. Journal of Insect Physiology 20, 1815–1823.

Feinsod, F.M., Spielman, A., 1980. Nutritional-mediated juvenile hormone secretion in mosquitoes. Journal of Insect Physiology 26, 113–117.

Flatt, T., Tu, M.P., Tatar, M., 2005. Hormonal pleiotropy and the juvenile hormone regulation of *Drosophila* development and life history. Bio Essays 27, 999–1010.

Fronstin, R.B., Hatle, J.D., 2008. A cumulative feeding threshold required for vitellogenesis can be obviated with juvenile hormone in lubber grasshoppers. Journal of Experimental Biology 211, 79–85.

Ferguson, E.M., Leese, H.J., 2006. A potential role for triglyceride as an energy source during bovine oocyte maturation and early embryo development. Molecular Reproduction and Development 73, 1195–1201.

Gilchrist, R.B., Lane, M., Thompson, J.G., 2008. Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. Human Reproduction Update 2, 159–177.

Gray, E.M., Bradley, T.J., 2003. Metabolic rate in female *Culex tarsalis* (Diptera: Culicidae): age, size, activity, and feeding effects. Journal of Medical Entomology 40, 903–911.

Greenplate, J.T., Glaser, R.L., Hagedorn, H.H., 1985. The role of factors from the head in the regulation of egg development in the mosquito *Aedes aegypti*. The Journal of Insect Physiology 31, 323–329.

Gwadz, R.W., Spielman, A., 1973. Corpus allatum control of ovarian development in *Aedes aegypti*. Journal of Insect Physiology 19, 1441–1448.

Hansen, I.A., Attardo, G.M., Park, J., Peng, Q., Raikhel, A.S., 2004. Target of rapamycin – mediated amino acid signaling in mosquito anautogeny. Proceedings of the National Academy of Sciences of the United States of America 101, 10626–10631.

Harshman, L.G., Zera, A., 2007. The cost of reproduction: the devil in the details. *Trends* in *Ecology* & *Evolution* 22, 80–86.

Highnam, K.C., Lusis, O., Hill, L., 1963a. The role of the corpora allata during oöcyte growth in the desert locust, *Schistocerca gregaria* Forsk. Journal of Insect Physiology 9, 587–590.

Highnam, K.C., Lusis, O., Hill, L., 1963b. Factors affecting oöcyte resorption in the desert locust *Schistocerca gregaria*. Journal of Insect Physiology 9, 827–837.

Hopwood, J.A., Ahmed, A.M., Polwart, A., Williams, G.T., Hurd, H., 2001. Malaria-induced apoptosis in mosquito ovaries: a mechanism to control vector egg production. Journal of Experimental Biology 204, 2773–2780.

Homa, S.T., Racowsky, C., McCaughey, R.W., 1986. Lipid analysis of immature pig oocytes. Journal of Reproduction and Fertility 77, 425–434.

Jalil, M., 1974. Observations on the fecundity of *Aedes triseriatus* Diptera: Culicidae. Entomologia Experimentalis Et Applicata 17, 223–233.

Koller, C.N., Raikhel, A.S., 1991. Initiation of vitellogenin uptake and protein synthesis in the mosquito (*Aedes aegypti*) ovary in response to a blood meal. Journal of Insect Physiology 37, 703–711.

Kokoza, V.A., Raikhel, A.S., 1997. Ovarian- and somatic-specific transcripts of the mosquito clathrin heavy chain gene generated by alternative 5-exon splicing and polyadenylation. Journal of Biological Chemistry 272, 1164–1170.

Kokoza, V.A., Snigirevskaya, E.S., Raikhel, A.S., 1997. Mosquito clathrin heavy chain: analysis of protein structure and developmental expression in the ovary during vitellogenesis. Insect Molecular Biology 6, 357–368.

Klowden, M.J., 1997. Endocrine aspects of mosquito reproduction. Archives of Insect Biochemistry and Physiology 35, 491–512.

Li, Y., Hernandez-Martinez, S., Unnithan, G.C., Feyereisen, R., Noriega, F.G., 2003. Activity of the corpora allata of adult female *Aedes aegypti*: effects of mating and feeding. Insect Biochemistry and Molecular Biology 33, 1307–1315.

Lea, A.O., Briegel, H., Lea, H.M., 1978. Arrest, resorption or maturation of oocytes in *Aedes aegypti*: dependence upon the quality of the blood meal and the interval between blood meals. Physiology Entomology 3, 309–316.

Leoni, G.G., Bebbere, D., Succu, S., Berlinguer, F., Mossa, F., Galioto, M., Bogliolo, L., Ledda, S., Naitana, S., 2007. Relations between relative mRNA abundance and developmental competence of ovine oocytes. Molecular Reproduction and Development 74, 249–257.

Matorras, R., Ruiz, J.I., Mendoza, R., Ruiz, N., Sanjurjo, P., Rodriguez-Escudero, F.J., 1998. Fatty acid composition of fertilization-failed human oocytes. Human Reproduction 13, 2227–2230.

Martin, D., Wang, S.F., Raikhel, A.S., 2001. The vitellogenin gene of the mosquito *Aedes aegypti* is a direct target of ecdysteroid receptor. Molecular and Cellular Endocrinology 173, 75–86.

Mermillod, P.P., Dalbies-Tran, R.R., Uzbekova, S.S., Thelie, A.A., Traverso, J., Perreau, C.C., Monget, P.P., 2008. Factors affecting oocyte quality: who is driving the follicle? Reproduction in Domestic Animals 43 (Suppl. 2), 393–400.

Mostowy, W.M., Foster, W.A., 2004. Antagonistic effects of energy status on meal size and egg-batch size of *Aedes aegypti* (Diptera: Culicidae). Journal Of Vector Ecology 29, 84–93.

Nagano, M., Katagiri, S., Takahashi, Y., 2006a. ATP content and maturational/developmental ability of bovine oocytes with various cytoplasmic morphologies. Zygote 14, 299–304.

Nagano, M., Katagiri, S., Takahashi, Y., 2006b. Relationship between bovine oocyte morphology and in vitro developmental potential. Zygote 14, 53–61.

Nayar, J.K., Sauerman Jr., D.M., 1975. The effects of nutrition on survival and fecundity in Florida mosquitoes. Part 2. Utilization of a blood meal for survival. Journal of Medical Entomology 12, 99–103.

Noriega, F.G., Wells, M., 1993. A comparison of three methods to isolate RNA from mosquitoes. Insect Molecular Biology 2, 21–24.

Raikhel, A.S., Dhadialla, T.S., 1992. Accumulation of yolk proteins in insect oocytes. Annual Review Entomology 37, 217–251.

Raikhel, A.S., Lea, A.O., 1983. Previtellogenic development and vitellogenin synthesis in the fat body of a mosquito: an ultrastructural and immunocytochemical study. Tissue and Cell 15, 281–299.

Raikhel, A.S., Lea, A.O., 1985. Hormone mediated formation of the endocytotic complex in mosquitoes. General and Comparative Endocrinology 57, 422–433.

Ramirez-Zacarias, J.L., Castro-Munozledo, F., Kuri-Harcuch, W., 1992. Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with oil red O. Histochemistry 97, 493–497.

Roth, T.F., Porter, K.R., 1964. Yolk protein uptake in the oocyte of the mosquito *Aedes aegypti*. Cell Biology 20, 313–332.

Sappington, T.W., Kokoza, V.A., Cho, W.L., Raikhel, A.S., 1996. Molecular characterization of the mosquito vitellogenin receptor reveals unexpected high homology to the *Drosophila* yolk protein receptor. Proceedings of the National Academies of Sciences 93, 8934–8939.

Sarfati, M., Krasnov, B.R., Ghazaryan, L., Khokhlova, I.S., Fielden, L.J., Degen, A.A., 2005. Energy costs of blood digestion in a host-specific haematophagous parasite. Journal of Experimental Biology 208, 2489–2496.

Schlaeger, D.A., Fuchs, M.S., 1974. Localization of dopa-decarboxylase in adult *Aedes aegypti* females. Journal of Experimental Zoology 187, 217–222.

Seo, S.J., Cheon, H.M., Sun, J., Sappington, T.W., Raikhel, A.S., 2003. Tissue- and stage-specific expression of two lipophorin receptor variants with seven and eight ligand-binding repeats in the adult mosquito. Journal of Biological Chemistry 278, 41954–41962.

Spielman, A., Gwadz, R.W., Anderson, W.A., 1971. Ecdysone-initiated ovarian development in mosquitoes. Journal of Insect Physiology 17, 1807–1814.

Sun, J., Hiraoka, T., Dittmer, N.T., Cho, K.H., Raikhel, A.S., 2000. Lipophorin as a yolk protein precursor in the mosquito, *Aedes aegypti*. Insect Biochemistry and Molecular Biology 30, 1161–1171.

Sturmey, R.G., Leese, H.J., 2003. Energy metabolism in pig oocytes and early embryos. Reproduction 126, 197–204.

Sturmey, R.G., Reis, A., Leese, H.J., McEvoy, T.G., 2009. Role of fatty acids in energy provision during oocyte maturation and early embryo development. Reproduction in Domestic Animals 44 (Suppl. 3), 50–58.

Soller, M., Bownes, M., Kubli, E., 1999. Control of oocyte maturation in sexually mature *Drosophila* females. Developmental Biology 208, 337–351.

Tadkowski, T.M., Jones, J.C., 1978. Endogenous synthesis of lipid yolk in mosquito oocytes. Experientia 34, 627.

Thomson, T.C., Fitzpatrick, K.E., Johnson, J., 2010. Intrinsic and extrinsic mechanisms of oocyte loss. Molecular Human Reproduction 16, 916–927.

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Terashima, J., Takaki, K., Sakurai, S., Bownes, M., 2005. Nutritional status affects 20-hydroxyecdysone concentration and progression of oogenesis in *Drosophila melanogaster*. Journal of Endocrinology 187, 69–79.

Tobe, S.S., Chapman, C.S., 1979. Effects of starvation and subsequent feeding on juvenile hormone synthesis and oocyte growth in *Schistocerca americana gregaria*. Journal of Insect Physiology 25, 701–708.

Troy, S., Anderson, W.A., Spielman, A., 1975. Lipid content of maturing ovaries of *Aedes aegypti* mosquitoes. Comparative Biochemistry and Physiology 50, 457–461.

Uchida, K., Moribayashi, A., 2002. Effects of physiological and unphysiological doses of 20-hydroxyecdysone on ovarian follicles in female *Culex pipiens pallens* (Diptera: Culicidae). Medical Entomology and Zoology 53, 153–161.

Uchida, K., Ohmorib, D., Eshita, Y., Oda, T., Kato, Y., Tomizawa, K., Moribayashi, A., Fukunaga, A., 1998. Ovarian development induced in decapitated female *Culex pipiens pallens* mosquitoes by infusion of physiological quantities of 20-hydroxyecdysone together with amino acids. Journal of Insect Physiology 44, 525–528.

Winckler, J., 1974. Vital staining of lysosomes and other cell organelles of the rat with neutral red. Progress in Histochemistry and Cytochemistry 6, 1–91.

Weaver, R.J., Pratt, G.E., 1981. Effects of starvation and feeding upon corpus allatum activity and oocyte growth in adult female *Periplaneta americana*. Journal of Insect Physiology 27, 75–83.

Woke, P.A., Ally, M.S., Rosenberger Jr., C.R., 1956. The numbers of eggs developed related to the quantities of human blood ingested in *Aedes aegypti*. Annals of the Entomological Society of America 49, 435–441.

Wrenzycki, C., Herrmann, D., Niemann, H., 2007. Messenger RNA in oocytes and embryos in relation to embryo viability. Theriogenology 68S, S77–S83.

Zera, A.J., Zhao, Z.W., 2004. Effect of a juvenile hormone analogue on lipid metabolism in a wing-polymorphic cricket: implications for the endocrine-biochemical bases of life-history trade-offs. Physiological Biochemical Zoology 77, 255–266.

Zhou, G., Pennington, J.E., Wells, M.A., 2004a. Utilization of pre-existing energy stores of female *Aedes aegypti* mosquitoes during the first gonotrophic cycle. Insect Biochemistry and Molecular Biology 34, 919–925.

Zhou, G.L., Flowers, M., Friedrich, K., Horton, J., Pennington, J., Wells, M.A., 2004b. Metabolic fate of [C-14]-labeled meal protein amino acids in *Aedes aegypti* mosquitoes. Journal of Insect Physiology 50, 337–349.

Ziegler, R., Ibrahim, M.M., 2001. Formation of lipid reserves in fat body and eggs of the yellow fever mosquito, *Aedes aegypti*. Journal Insect Physiology 47, 623–627.

Ziegler, R., 1997. Lipid synthesis by ovaries and fat body of *Aedes aegypti* (Diptera: Culicidae). European Journal of Entomology 94, 385–391.