

Starvation affects vitellogenin production but not vitellogenin mRNA levels in the lubber grasshopper, *Romalea microptera*

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Abstract

The interaction of juvenile hormone (JH) and nutrition was studied during the oviposition cycle of the Eastern Lubber grasshopper (*Romalea microptera*). Starvation of females early or in the middle of the cycle inhibited oocyte growth. Starvation for 4 days also reduced hemolymph levels of JH III and vitellogenesis (Vg) to 25% and 15%, respectively, of the levels in fed animals. Likewise, Vg production by fat body fragments incubated *in vitro* was reduced to 2% of the levels in fed animals and total protein synthesis was reduced to 25%, suggesting that starvation had a stronger effect on Vg synthesis than on protein synthesis. These effects were reversed when starved animals were fed again. However, fat body levels of Vg-mRNA were similar in fed and starved animals, indicating that starvation did not affect transcript levels. We tested whether the decline in JH levels mediated the other starvation effects by infusing animals with JH III or vehicle for 2 days at the onset of starvation. Infusion of JH elevated JH and Vg-mRNA levels 670% and 103%, respectively, above the levels in vehicle-infused animals. However, Vg production and hemolymph levels of Vg were similar to the levels in vehicle-infused animals. These data suggest that JH alone is insufficient to stimulate Vg production.

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1. Introduction

The reproductive output of many insects is strongly affected by adult nutrition (Engelmann, 1970; Nijhout, 1994; Wheeler, 1996; Moehrli and Juliano, 1998). Some of the most striking examples of this are found in *Rhodnius prolixus* (Davey, 1993) and many mosquitoes (Hagedorn, 1994) where adult feeding is a necessary prerequisite for egg production. In these insects, feeding above a threshold level initiates a cascade of endocrine and cellular events leading to oviposition. The role of

adult nutrition has also been extensively studied in cockroaches and locusts, where both the quantity (Highnam et al., 1966; Kunkel, 1966; Bell, 1971) and the quality (McCaffery, 1975; Lee and Wong, 1979; Woodhead and Stay, 1989; Schal, et al., 1993) of adult nutrition affect reproduction.

Hormones are thought to mediate the effects of nutrition on reproduction, though the specific compounds and their roles in regulating reproduction are not completely understood. Nevertheless, it is clear that juvenile hormone (JH) often plays important roles in insect reproduction, and in many species JH acts as a gonadotropin by stimulating both the production of vitellogenin (Vg; the major yolk protein in eggs) (Engelmann, 1983) by the fat body and regulating Vg uptake by oocytes (Abu-Hakima and Davey, 1977; Wyatt and Davey, 1996). JH also appears to play a role

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in mediating the effects of nutrition on reproduction (Nijhout, 1994; Ringo, 2002). Thus, starvation of a number of species, including *Schistocera americana* (Tobe and Chapman, 1979), *Periplaneta americana* (Weaver and Pratt, 1981), and *Blattella germanica* (Schal et al., 1993), causes a decrease in JH production by corpora allata (CA) and an inhibition of oocyte growth. Refeeding of these animals leads to an increase in JH production by the CA and the resumption of oocyte growth. These and other results support a model of reproductive control in which nutrition increases hemolymph levels of JH, which in turn increase Vg production and its uptake by oocytes as they mature.

In the Eastern Lubber grasshopper, *Romalea microptera*, the reproduction cycle of virgin females fed ad libitum (~35 days for animals from our colony) is divided into a somatic growth phase followed by a reproductive growth phase. The body mass of these animals increases ~80% during the somatic growth phase (~13–15 days). Primary oocytes are not vitellogenic for about the first 9 days, after which they begin to import vitellogenin and grow (Sundberg et al., 2001). These processes appear to be controlled by underlying endocrine events. One hormone that is clearly involved is JH III, the JH present in lubber grasshoppers (Li et al., 2005). JH levels rise during the oviposition cycle and this rise is closely paralleled by increases in fat body levels of Vg-mRNA and hemolymph levels of Vg (Borst et al., 2000).

Lubber reproduction is strongly affected by the level of adult nutrition. Switching animals from an ad libitum to a severely restricted diet at the adult molt or early in the first oviposition cycle increases the time to oviposition and reduces the number of eggs laid (Moehrlin and Juliano, 1998; Hatle et al., 2001). These morphological events are correlated with endocrine events. Grasshoppers switched to a restricted diet at the adult molt show a delay in the rise of their hemolymph levels of JH and Vg, suggesting that the increased time to oviposition in these animals is due in part to an increase in the duration of their somatic growth phase (Hatle et al., 2001). In addition, under normal conditions females usually resorb some of their developing oocytes. However, starvation dramatically increases the percentage of oocytes resorbed (Sundberg et al., 2001).

In this paper, we examine the effects of starvation on the lubber grasshopper and how these effects are mediated by its endocrine system. We first characterize oocyte development in fed and starved females, and demonstrate that acute starvation causes a rapid termination of oocyte growth. We then examine the effects of starvation on hemolymph levels of Vg and JH, Vg production by fat body fragments incubated in vitro, and fat body levels of Vg-mRNA. Finally, we test the hypothesis that JH mediates the effects of starvation on

oocyte growth. We predicted that starvation would decrease hemolymph levels of JH, and that these lower levels of JH would cause a decline in Vg production by the fat body and Vg uptake by the oocytes. As a result, oocyte growth would cease. Conversely, we predicted that the infusion of JH III into starved animals would maintain the production of Vg and its uptake by oocytes, thereby allowing oocyte growth to continue. Our results show that starvation does decrease hemolymph levels of JH and Vg production, leading to decreased hemolymph levels of Vg. However, starvation had no effect on Vg-mRNA levels. Likewise, JH treatment increased Vg-mRNA levels but did not stimulate Vg production and appeared to have no effect on its uptake. These results suggest that the production of Vg and its uptake by the oocytes are regulated by an additional factor(s) that is affected by the level of nutrition.

2. Methods

2.1. Animals

The female Eastern Lubber grasshoppers, *Romalea microptera* (Beauvois), used in these studies came from our laboratory colony which was established in 1996–97 with individuals collected near Copeland, FL, USA. On the day of the adult molt, females were separated from males and placed in an environmental chamber with a 14L:10D photoperiod and a corresponding 32:24 °C thermocycle. We used only virgin females, which require ~35 days to complete their first oviposition cycle at this temperature regime (Walker et al., 1999).

We first determined the effects of starvation on oocyte growth and resorption. Newly eclosed adult females were housed in group cages and fed Romaine lettuce, oatmeal, and wheat bran ad libitum (Matuszek and Whitman, 2002). Each animal was assigned to one of three groups. Females in the *fed* group remained in the group cages and continued to receive food until analyzed. Animals in the *starved, d 10* and *starved, d 20* groups were transferred on days 10 or 20, respectively, to individual 0.5 plastic cages and thereafter received no food but were provided with water daily. We dissected five to seven females from each of the three groups at 5-day intervals and measured the lengths of five primary oocytes and the percentage of 1° oocytes that were in the process of resorption, according to Sundberg et al. (2001). We also examined five well-fed females on day 32.

In our second study, we examined the hormonal mechanisms mediating reproduction during starvation. Newly eclosed females were housed in group cages and fed Romaine lettuce and oatmeal ad libitum. On day 17, the animals were transferred to individual 0.51 plastic

cages. We chose day 17 for this transfer because it is midway through the ~35-day oviposition cycle for virgins and ~7 days after the onset of vitellogenesis (Sundberg et al., 2001). Day 17 also falls within the period of maximum oocyte growth (Fig. 1), and when hemolymph levels of Vg are still rising (Borst et al., 2000). Finally, it has been previously shown that the time of oviposition in animals fed a reduced diet (but not starved) is determined by day 17, but the number of eggs that they will produce has not (Moehrli and Juliano, 1998). Each animal was assigned to one of three groups. Animals in the *fed* group continued to receive food ad libitum. Females in the *starved* group received no food but were provided with water daily. Animals in the *starved/re-fed* group were starved for 2 days and then fed for 2 days. On days 17, 19 and 21 after the adult molt, hemolymph samples were taken from the animals to determine the levels of Vg and JH. In addition, we dissected seven animals from each group on each of these days and measured the lengths of five primary oocytes. Fat body tissue was removed and used to determine Vg production and total protein synthesis in vitro as well as the level of Vg-mRNA.

In a third study, we determined the effect of JH infusion on reproduction. Animals were maintained as in the second study. On day 17, animals were fitted with mini-osmotic pumps and infused with a solution of 10R-JH III or the vehicle alone. These animals were analyzed as above after 2 days.

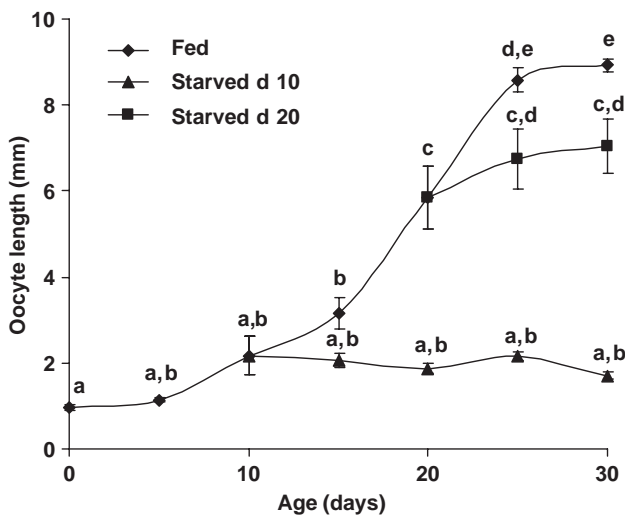


Fig. 1. Starvation inhibited oocyte growth. Virgin females were placed on an ad libitum diet on the day of the adult molt. *Fed* animals were maintained on this diet throughout the experiment while *Starved d 10* and *Starved d 20* were given water but no food from days 10 and 20, respectively. On the indicated days, 5–7 animals in each treatment group were dissected and the length of five primary oocytes determined (mean \pm SEM). Means with different letters are significantly different ($P < 0.05$; ANOVA).

2.2. Materials

Radioactive [^3H]-juvenile hormone III (17.5 Ci/mmol) and [^3H]-leucine (42.5 Ci/mmol) were purchased from Perkin Elmer Life Sciences (Boston, MA). Most other chemicals, including nonradioactive JH III and Grace's Insect Medium, were purchased from Sigma Chemical Co (St. Louis, MO). PCR primers were purchased from Integrative DNA Technologies (Coralville, IA) and the osmotic minipumps (model 2002) were purchased from Durect Corp (Cupertino, CA).

2.3. Hemolymph levels of JH

Hemolymph samples (10 μl) were placed in 0.5 ml acetonitrile and held at -20°C . These samples were analyzed for JH III by RIA (Borst et al., 2000). 10R-JH III and [^3H] 10R-JH III were separated from racemic [^3H]-JH III and unlabeled JH by HPLC (Cusson et al., 1997) and used as the RIA standard and radioligand, respectively.

2.4. Measurement of Vg

Hemolymph samples (1 μl) were placed in 50 μl of hemolymph buffer (50 mM Tris, pH 7.5 with 100 mM NaCl, 1 mM EDTA, 0.1 mM dithiothreitol, 0.15% Tween 20, 10 $\mu\text{g}/\text{ml}$ leupeptin, and 10 $\mu\text{g}/\text{ml}$ phenylmethylsulfonyl fluoride and stored at -20°C . Culture medium from fat body incubation was collected and stored at -20°C . The samples were analyzed for Vg with a sandwich ELISA (Borst et al., 2000) using a streptavidin-POD conjugate (Roche Diagnostics, Indianapolis, IN). The Vg standard was purified from hemolymph using anion exchange chromatography. The peak fractions (identified with the Vg-ELISA) were pooled and the protein content determined with the BioRad protein assay (BioRad, Richmond, CA).

2.5. In vitro analysis of protein synthesis and Vg production by the fat body

Fat body tissue was removed from each animal, rinsed in sterile Ringer's solution (NaCl 100 mM, K_2SO_4 10 mM, MgSO_4 10 mM, CaCl_2 3.5 mM, glucose 10 mM, glycine 10 mM, HEPES 2.5 mM, pH 7.2), and divided into small fragments (<2 mm in the largest dimension). Two or three fragments were transferred into each of two wells of a 24-well culture plate containing 400 μl of Grace's culture medium (pH 6.6) modified with sufficient sucrose to increase the osmolality to 380 mOsm (the osmolality of lubber hemolymph) and gentamycin (50 $\mu\text{g}/\text{ml}$). After a 2 h preincubation at room temperature, the tissue was transferred to a well containing modified Grace's medium supplemented with ^3H -leucine (10 $\mu\text{Ci}/\text{well}$, final specific activity in

the medium = 44 mCi/mmol). After incubation for 4 h at room temperature on an orbital shaker (100 rpm), the tissue fragments were removed from the culture medium, washed with cold Ringer's solution, weighed, and homogenized in 0.1% bovine serum albumin (BSA). Total protein synthesis was determined by mixing an aliquot of the homogenate with 2 mg of IgG and precipitating the protein with an equal volume of ice-cold 10% TCA. The pellet was washed with cold 5% TCA, solubilized, and the radioactivity measured with a liquid scintillation counter.

To measure Vg production during the *in vitro* incubation, we determined the Vg released into the culture medium during the second incubation period by ELISA (Martín et al., 1995). In preliminary experiments (not shown), we demonstrated that Vg immunoactivity in the culture medium increased linearly with incubation time (up to 72 h) and mass of fat body tissue. In addition, all of the immunoactive material present in the incubation medium eluted from an anion exchange column (NaCl gradient of 0–1 M in 50 mM Tris, pH 8.0) in the same fractions as hemolymph vitellogenin. Furthermore, incubation of fat body fragments with puromycin (40 µg/ml) during the first and second incubation periods decreased the amount of Vg in the second incubation medium by 80%. Thus, the Vg detected during the second incubation was largely produced by the tissue during the incubation, and was not merely hemolymph Vg transferred with the tissue. Finally, the levels of Vg produced *in vitro* seem reasonable in view of the amount of Vg found in the hemolymph. Day 17 animals produced about 3 µg Vg/mg fat body/hr. Animals at this stage contain over 300 mg of fat body (Whitman, unpublished observations), so the total estimated production of Vg would be over 22 mg/day. Likewise, the total hemolymph volume of females during the middle of their oviposition cycle is about 2.2 ml (Li and Borst, unpublished) and Vg levels on day 17 are about 12 mg/ml. Thus, total hemolymph Vg (26 mg) on day 17 is similar to the *in vitro* estimates of Vg synthesis.

2.6. Quantification of Vg-mRNA

Fat body tissue was homogenized in TriReagent and extracted with chloroform. After centrifugation, the RNA in the aqueous supernatant was precipitated with isopropanol. The RNA pellet was dissolved in DEPC-water and stored in ethanol at -80°C . For the quantification of Vg-mRNA, the RNA suspension from each animal was vortexed, an aliquot was removed and centrifuged, and the pellet dried. The RNA was dissolved in DEPC-treated water and quantified by absorbance (OD_{260}). A second aliquot containing 0.4 µg of the RNA suspension was centrifuged, the pellet resuspended in DEPC-water and used to produce cDNA

by reverse transcription (0.5 mM dNTP, 50 mM Tris pH 7.5, 75 mM KCl, 3 mM MgCl_2 , 10 mM DTT, 0.1 µg oligo random hexamer (Promega) and 20 units of MMLV reverse transcriptase, 37°C for 1 h). These cDNA preparations were analyzed by real-time PCR (total reaction volume = 20 µl). An aliquot (2 µl) of each cDNA was added to 3 fmol each of a primer pair for lubber Vg-mRNA (forward 5'-GTACGTCATGGCTGTTGGTC-3'; reverse 5'-GGAAGTATTCTGCGCTGCTT-3') along with 5 µl of SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). Parallel reactions using aliquots of these cDNA samples with the primer pair for lubber β -actin (GenBank # AY331667 forward 5'-CTTACTGAGAGAGGCTACAG-3'; reverse 5'-actt-gagggaagcagcagttg-3') were performed to determine the levels of β -actin-mRNA. Standard curves were made by analyzing serial dilutions of known amounts of Vg and β -actin PCR fragments (synthesized from fat body cDNA using the above primer pairs followed by gel purification and quantification at OD_{260}). Each sample or standard was analyzed in triplicate. The average Ct value (number of PCR cycles needed to reach a threshold amount of product) was calculated for each dilution of the Vg and β -actin standards and for both Vg and β -actin mRNA in each sample. The average Ct values of the standards were used to create a standard curve, and the average CT value of each sample was compared to the appropriate standard curve to determine the quantity of Vg and actin mRNA in each sample. The amount of Vg-mRNA was then divided by the amount of β -actin-mRNA to determine the Vg-mRNA/ β -actin-mRNA ratio.

2.7. JH infusion

Animals were infused with JH using 0.2 ml Alzet osmotic minipumps. Each minipump was filled with 10R-JH III (isolated as described above, 150 µg/ml) in ethanol and 0.9% NaCl (1:1). Other pumps were filled with the vehicle alone. The pumps were activated by soaking overnight in 0.9% NaCl at 37°C and then sealed in a small plastic reservoir containing 2 ml distilled water. Using this procedure, these pumps delivered 1 µl/h for up to 7 days. The pump outlet was connected with Tygon tubing (ID = 0.01") to a 30 gauge needle inserted into the body cavity through the dorsal abdominal carapace and cemented to the exoskeleton with dental resin. The pump/reservoir was tied to the back of the grasshopper with parafilm. Infused animals were housed individually in plastic containers as described above.

2.8. Statistical analysis

The results of the first two experiments were analyzed by ANOVA (Kruskal–Wallis) followed by Dunn's

multiple comparison test. The results of the third experiment were analyzed by Student's *t*-test.

3. Results

3.1. Oocyte growth in fed and starved females

Females fed ad libitum had little oocyte growth during the first 10 days after the adult molt (Fig. 1); oocyte lengths were not statistically different from those of oocytes on day 0 ($P>0.05$). Day 10 oocytes were translucent and contained only a few yellow granules, indicating that they had just begun to accumulate vitellogenin. However, day 15 oocytes were significantly larger ($P<0.05$) than day 0 oocytes and were opaque and bright yellow, indicating that they were rapidly growing and accumulating Vg. By day 30, the oocytes had reached their final size of ~ 9 mm, which was 800% longer than oocytes on day 0 ($P<0.001$). Oocytes were ovulated into the lateral oviducts on \sim day 32, and were laid on \sim day 35. Oocyte resorption was staggered, and the number of oocytes being resorbed increased between days 10–32, peaking on day 32 at about 18%. There was no visible resorption of oocytes before the onset of vitellogenesis (~ 10 days).

In contrast to fed animals, females starved beginning on days 10 or 20 showed no significant oocyte growth after starvation ($P>0.05$; Fig. 1), suggesting that starvation rapidly terminates oocyte growth. Likewise, the length of oocytes from animals starved beginning on day 20 were not longer over the next 10 days than the oocytes on day 20 ($P>0.05$). Thus, on both days there was little if any growth of oocytes after the onset of starvation. In both day 10 and day 20 starved animals, the resorption rates of primary oocytes increased with time, and by day 30 reached 100% in animals starved on day 10 and 46% in animals starved on day 20.

3.2. Starvation caused a decline in hemolymph levels of JH and Vg

Adult females were fed for 17 days and then placed in one of three groups. Animals in the *fed* group were fed for an additional 2 or 4 days, those in the *starved* group were not fed for the next 2 or 4 days, and those in the *starved-refed* group were not fed for 2 days and then fed for 2 days. The length of the oocytes in fed animals increased during the 4-day period and were significantly larger ($P<0.01$) on day 21 than on day 17 (see Fig. 2A). While the observed change in oocyte length during this period may seem modest (1.25 mm), small changes in oocyte length (a linear measure) reflect more substantial changes in oocyte volume. In starved animals, oocyte length showed no significant change during the experi-

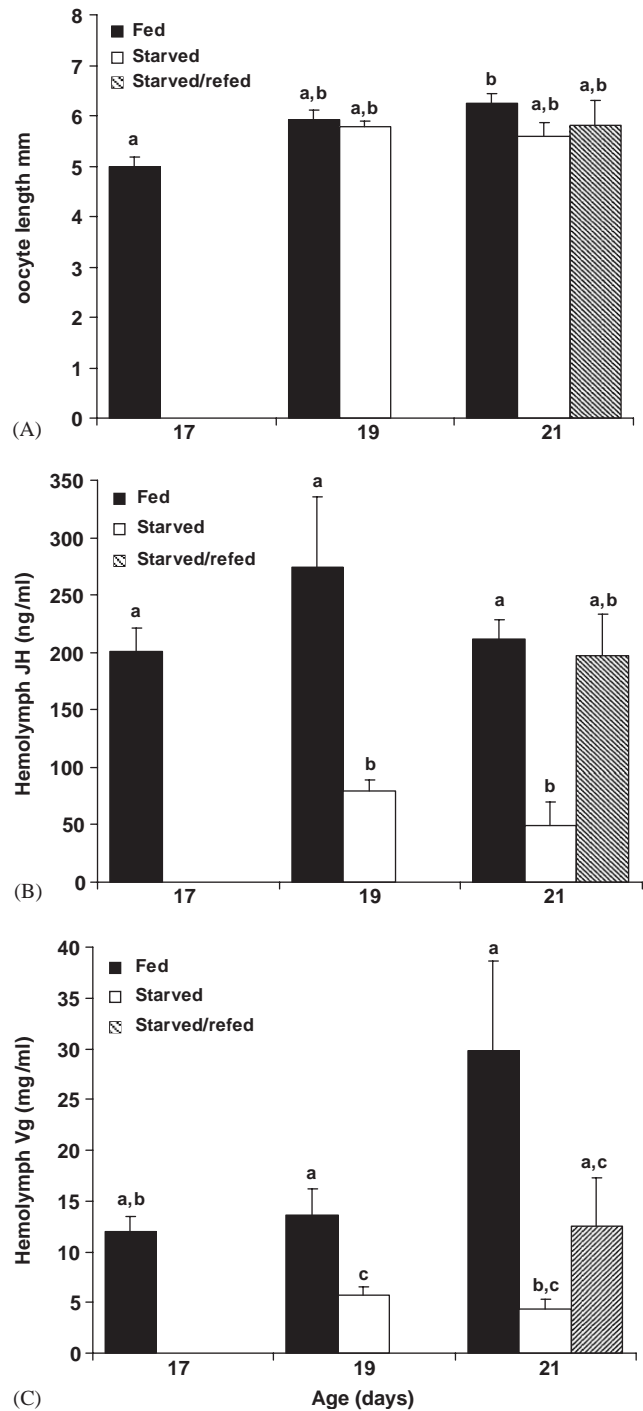


Fig. 2. Starvation inhibited oocyte growth and decreased hemolymph levels of JH III and vitellogenin (Vg). These effects were reversed when animals were refed. For this and Figs. 3 and 4, females were fed ad libitum until day 17 and then divided into three groups. *Fed* animals were maintained on the ad libitum diet, *starved* animals were given water but no food, and *starved-refed* animals were starved for 2 days and then fed for 2 days. (A) Oocyte growth is inhibited by starvation. (B) Hemolymph levels of JH (determined by RIA). (C) Hemolymph levels of Vg (determined by ELISA). Columns (mean \pm SEM) with different letters are significantly different ($P<0.05$; ANOVA).

ment ($P > 0.05$). Likewise, oocyte length in starved–refed animals (starved for 2 days and then fed for 2 days) was similar to the length of oocytes on day 17.

The inhibition of oocyte growth caused by acute starvation was accompanied by a decrease in hemolymph levels of JH III (Fig. 2B). In fed animals, hemolymph levels of JH after 2 and 4 days (days 19 and 21) were similar ($P > 0.05$) to the initial levels. The JH levels measured on these days were similar to those reported previously in lubber grasshoppers at this phase of the oviposition cycle (Borst et al., 2000). In contrast, JH levels in starved animals fell rapidly and were significantly reduced after 2 days ($P < 0.001$). By 4 days (day 21), JH levels in starved animals had decreased to 24% of levels observed on day 17 ($P < 0.01$). The JH levels of starved–refed animals on day 21 were similar to the levels measured on day 17 and day 21 in fed animals ($P > 0.05$).

Starvation also affected hemolymph levels of Vg (Fig. 2B). Vg levels rose modestly in fed animals, and after 4 days (day 21) were 150% above the initial levels on day 17, though this was not statistically significant ($P > 0.05$). In starved animals, hemolymph levels of Vg decreased, and after 4 days were 36% of initial levels on day 17 ($P > 0.05$) and 15% of levels observed in fed animals on day 21 ($P < 0.01$). The Vg levels in starved–refed animals on day 21 were nearly identical to the initial levels ($P > 0.05$) and intermediate between the levels observed in fed and starved animals on day 21 ($P > 0.05$).

3.3. Starvation selectively decreased Vg production by the fat body

The effect of starvation on oocyte growth and hemolymph levels of Vg suggested that Vg production by the fat body is lower in starved animals. Therefore, we incubated fragments of fat body tissue in vitro and measured their production of Vg (Fig. 3A). In fed animals, the levels of Vg production after 2 and 4 days (days 19 and 21) changed little from the initial levels measured on day 17 ($P > 0.05$). However, starvation caused Vg production by fat body tissue to fall to 46% after 2 days (day 19) and 1.5% after 4 days (day 21) of the levels in fed animals on these days ($P > 0.05$ and < 0.01 , respectively). This decrease in Vg production rate was the most pronounced change we observed upon starvation. Vg production by fat body from starved–refed animals was intermediate to the levels observed in starved and fed animals on day 21.

The sharp decline in Vg production observed in starved animals could be due to an overall decline in protein synthesis. Therefore, we also measured the incorporation of [3 H]-leucine into total protein by these fat body fragments (Fig. 3B). In fed animals, total protein synthesis rose to a level on day 21 that was 75%

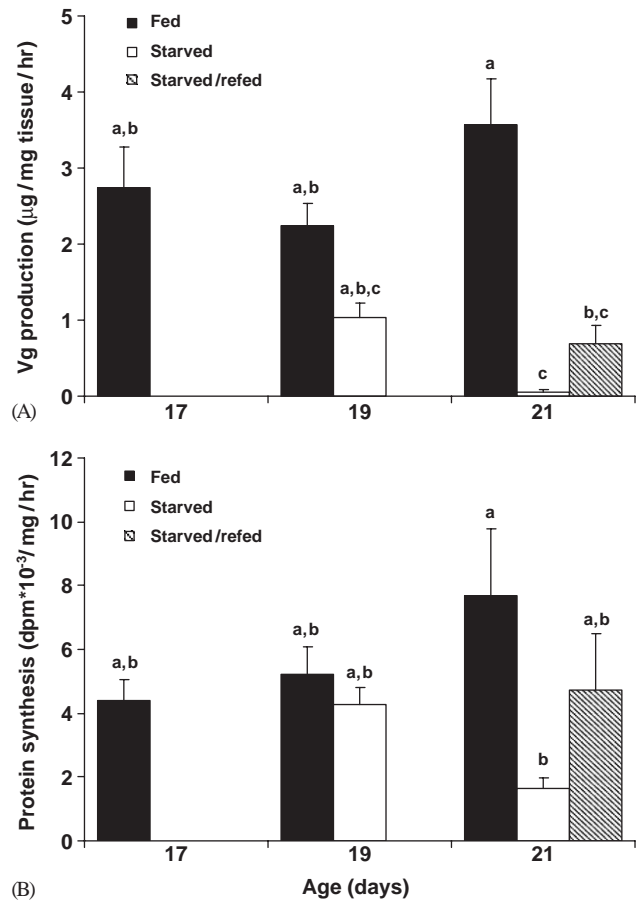


Fig. 3. Starvation decreased Vg production and total protein synthesis by fat body fragments incubated in vitro. These effects were reversed when animals were refed. (A) Vg production (μg Vg released into the culture medium per mg tissue per hour) was determined by ELISA. Columns (mean \pm SEM) with different letters are significantly different ($P < 0.05$; ANOVA). (B) Total protein synthesis ($\text{dpm} \times 10^{-3}/\text{mg}$ tissue per hour) was determined by incubating fat body fragments with [3 H]-leucine for 4 h and measuring the incorporation of radiolabel into TCA-precipitable material. Columns (mean \pm SEM) with different letters are significantly different ($P < 0.05$; ANOVA).

above the incorporation measured on day 17, though this was not statistically significant. In starved animals, total protein synthesis was reduced to 82% on day 19 and 21% on day 21 of the incorporation in fed animals on these days ($P > 0.05$ and < 0.05 , respectively). Total protein synthesis in starved–refed animals was intermediate to the levels observed in starved and fed animals on day 21.

3.4. Starvation had no effect on Vg-mRNA levels

The decrease in Vg production during starvation could reflect a change in the level of Vg-mRNA (e.g., by changing the rate of its transcription or its degradation). Therefore, we quantified Vg-mRNA levels in fat body tissue by real time PCR and normalized these levels to total RNA content or the level of β -actin mRNA. Both

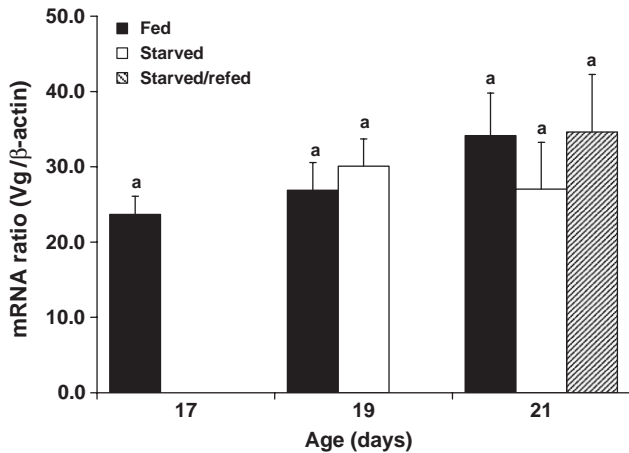


Fig. 4. Starvation had no effect Vg-mRNA levels. Fat body tissue was analyzed by real time-PCR to determine the levels of Vg-mRNA and β -actin-mRNA. Results are expressed as the ratio of these 2 mRNAs. Columns (mean \pm SEM) with different letters are significantly different ($P < 0.05$; ANOVA).

calculations gave similar results, and the latter are shown here (Fig. 4). In fed animals, the ratio of Vg-mRNA to β -actin-mRNA on day 21 was increased 44% above the level measured on day 17. Likewise, starved animals had a 14% increase on day 21, and starved-refed animals had an increase of 46%. None of these changes was statistically significant.

3.5. JH infusion did not stimulate vg production in starved animals

The rapid decrease in JH levels after the onset of starvation might be responsible for the decrease in fat body production of Vg, leading to a decrease in hemolymph levels, Vg uptake, and ultimately the cessation of oocyte growth. We tested this hypothesis directly by treating animals at the onset of starvation with JH for 2 days. Because JH has a short half-life in vivo (6–8 h; (Knepp et al., 1999), we infused animals with JH or vehicle only (controls) for 2 days (Fig. 5). Infusion of animals increased their JH levels 670% above the levels measured in control animals ($P < 0.01$). Hemolymph levels of Vg in JH-infused animals were 64% above those of the control animals, but this increase was not significant ($P > 0.05$). The in vitro production of Vg by fat body tissue from JH-infused animals was slightly reduced to 85% of the levels in control animals, but this effect was also not significant ($P > 0.05$). In contrast, the ratio of Vg-mRNA to β -actin-mRNA rose significantly to 103% above the ratio measured in control animals ($P < 0.05$). It should be noted that the Vg to β -actin mRNA ratios of control animals (Fig. 5) were only 40% of those observed in starved animals on day 19 (Fig. 4). It seems likely that this difference is related, in part, to some aspect of the

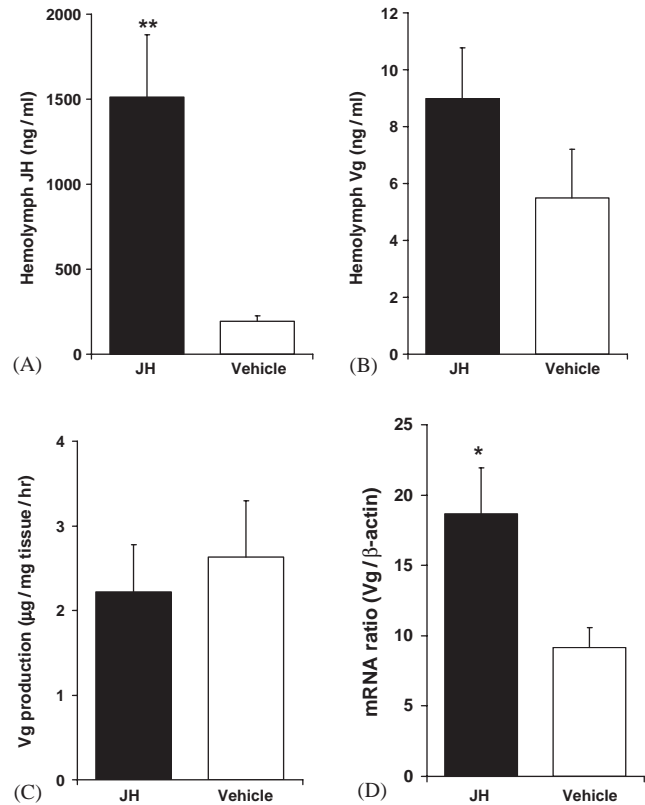


Fig. 5. The treatment of starved animals with JH III elevated Vg-mRNA levels but did not affect Vg production or hemolymph levels. Animals were infused for 2 days at the onset of starvation (day 17) with 1 μ l/h of either 10R JH III (150 μ g/ml) or vehicle (Ringer's: ethanol, 1:1). JH levels (A) and Vg levels (B) in the hemolymph were measured by RIA and ELISA, respectively, Vg production (C) was determined by incubating fat body fragments in vitro, and Vg-mRNA levels (D) were determined by real-time PCR. Columns show the means (\pm SEM). * $P < 0.05$; ** $P < 0.01$ (t -test).

infusion procedure (e.g., the presence of the infusion needle/apparatus or the use of 50% ethanol as the infusion solvent).

4. Discussion

Our results show that nutrition has important and complex effects on oocyte growth in the adult lubber grasshopper. Starvation of animals either at the onset of vitellogenesis (day 10) or in the middle of vitellogenesis (day 17 or 20) caused a rapid cessation in oocyte growth, which was detected in as little as 4 days after the onset of starvation. Starvation of lubber females also led to an increase in the resorption of oocytes. These results suggest that lubbers possess a flexible reproductive physiology that can respond quickly to changing environmental conditions. Similar results have been reported for other hemimetabolous insects (e.g., Highnam et al., 1966; Kunkel, 1966; Tobe and Chapman, 1979; Weaver and Pratt, 1981).

The effects of starvation on oocyte growth were correlated with changes in the cellular and molecular processes of vitellogenesis. The most striking effect of starvation was on Vg production by the fat body, which declined to very low levels after 4 days. Starvation also decreased total protein synthesis by the fat body, but this effect was neither as rapid nor as large as the effect of starvation on Vg production. Hence, starvation affects Vg production more strongly and perhaps selectively. It seems likely that the precipitous decline in Vg production is a major reason for the difference in hemolymph Vg levels of starved and fed animals on day 21. The lower levels of Vg in starved animals would, in turn, have an obvious effect on the growth of their oocytes, since the accumulation of Vg is a major factor affecting the growth of oocytes during vitellogenesis.

Vg levels also fall during the latter phase (days 23–33) of the lubber oviposition cycle when the primary oocytes are nearing maturity (Borst et al., 2000). However, this decline appears to be fundamentally different from the decline that occurs during starvation. Vg-mRNA levels did not change significantly in either fed or starved animals during the course of this experiment (days 17–21). Thus, the decline in Vg production and hemolymph Vg levels induced by starvation appear to involve post-transcriptional mechanisms (e.g., changes in Vg-mRNA translation). In contrast, Vg-mRNA levels decrease toward the end of the oviposition cycle, falling from a maximum on day 23 to ~27% of that level on day 33 (Borst et al., 2000). This decline in Vg-mRNA at the end of the oviposition cycle is correlated with decreased hemolymph levels of Vg and JH, which by day 33 have dropped to 18% and 44%, respectively, of their maximum levels (Borst et al., 2000). Taken together, these results suggest that Vg production can be regulated in several ways, including changes in Vg-mRNA levels (observed at the beginning and the end of the oviposition cycle) and post-transcriptional mechanisms (such as those disclosed in this study by starvation).

Starvation caused a rapid fall in hemolymph levels of JH in the lubber grasshopper. Indeed, the level of JH was the first characteristic to show a significant change after the onset of starvation. Likewise, JH levels rose quickly when starved animals were refed. These results show that JH levels are very sensitive to acute starvation, similar to observations made in other species, including *S. americana* (Tobe and Chapman, 1979), *P. americana* (Weaver and Pratt, 1981), and *B. germanica* (Schal et al., 1993).

The observation that starvation caused a decline in JH levels and that Vg production dropped shortly thereafter initially seemed to support the suggestion that JH acts as an intermediate between nutrition and Vg production. Indeed, such a relationship appears to exist in some species (e.g., *P. americana* and *B. germanica*) in which vitellogenesis can be reinitiated in starved

individuals by treatment with JH analogues (Bell, 1971; Schal et al., 1993). Nevertheless, we found that the infusion of 10R-JH III into starving lubber grasshoppers did not increase either Vg production or hemolymph levels of Vg. However, the lack of an effect on Vg production is not due to JH resistance of the fat body, since the infusion of JH doubled the level of Vg-mRNA in this tissue. The reason that lubber grasshoppers respond differently to JH treatment than *P. americana* and *B. germanica* is not clear. However, it may reflect differences in the nutritional reserves available in these species to continue reproduction during starvation. In any case, JH insufficiency does not appear to be solely responsible for the starvation-induced decline in vitellogenesis in lubber grasshoppers. Rather, Vg production appears to be regulated by multiple factors in this species. Thus, JH may regulate the production of Vg-mRNA and another factor(s) may regulate the translation of Vg-mRNA. There are several possible reasons that Vg-mRNA levels remain elevated during starvation when JH levels decreased. However, we suspect that the most likely explanation is that Vg-mRNA has a long half-life during the middle of the oviposition cycle.

Considerable evidence supports the existence of several factors that modulate vitellogenin production by the fat body (Bellés, 1998). In *Locusta migratoria*, adipokinetic hormone inhibits the production of Vg by the fat body and appears to be responsible for the fall in Vg production at the end of the oviposition cycle (Moshitzky and Applebaum, 1990; Glinka et al., 1995). Allatostatins appear to directly inhibit vitellogenin production by fat body tissue in *B. germanica*, possibly by inhibiting glycosylation (Martin et al., 1996). Brain extracts from *L. migratoria* stimulate Vg synthesis by fat body tissue incubated in vitro (Glinka et al., 1995). Finally, a neuropeptide isolated from the corpora cardiaca of *L. migratoria* (ovary maturing parsin, Lom-OMP) has been shown to stimulate oocyte development (Girardie et al., 1992). This peptide stimulates ecdysteroid production in the ovary, which then increases fat body production of Vg (Girardie and Girardie, 1996). It also appears to have an additional stimulatory role on the fat body (Girardie et al., 1998). If a compound related to Lom-OMP exists in the lubber grasshopper, this latter action is probably more important, since hemolymph ecdysteroids have no detectable role in regulating vitellogenin in production this species (Hatle et al., 2003).

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