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Gonadal ecdysone titers are modulated by protein availability but do not impact protein appetite

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ABSTRACT

How animals survey internal nutrient availability to modulate specific appetites is currently largely unknown. Dietary proteins have a profound impact on the reproductive capacity and the selection of food sources in insects. When deprived of dietary proteins, insects stop producing eggs and develop strong protein appetites. In many adult insects, the ovaries are the site of synthesis of the ecdysone hormone. Therefore, an attractive hypothesis is that protein availability changes the gonadal production of ecdysone, which instructs the brain to increase its preference for yeast. We combine quantitative feeding assays, dietary manipulations, hormonal measurements, and genetic germline manipulations to test this hypothesis in *Drosophila melanogaster*. Our results show that upon yeast deprivation mated adult female *Drosophila* develop a strong yeast appetite and strongly reduce their egg production. This dietary manipulation also leads to a drastic reduction in ecdysone titers. However, the drop in ecdysone is not linked to the increase in yeast appetite as mutants with impaired oogenesis are able to adapt yeast intake to their nutrient state while displaying a constitutive low ecdysone titer. Interestingly, a low ecdysone titer is correlated with a lower level of overall food intake. Our data therefore show that in mated females the level of ecdysone reflects the level of protein in the diet and the physiological state of the ovaries. While the ovaries and ecdysone are unlikely to instruct the brain to develop a yeast appetite upon protein deprivation, they seem to be able to control overall food intake.

1. Introduction

Animals are constantly challenged to make decisions throughout their lifespan. In particular, feeding decisions are of paramount importance for the organism as the adequate supply of energy and other nutrients ensures its survival and reproduction. Homeostasis is a property of complex organisms allowing them to adapt to environmental fluctuations in the availability of resources (Leopold and Perrimon, 2007; Rajan and Perrimon, 2011). Accordingly, the coordination of nutrient intake and utilization is key to homeostasis and animals have evolved diverse behavioral repertoires to maintain adequate levels of nutrients (Corrales-Carvajal et al., 2016; Simpson and Raubenheimer, 2012).

Reproduction is highly dependent on nutrition, in particular, on amino acid (AA) availability (Drummond-Barbosa and Spradling, 2001; Hansen et al., 2004; Hosios et al., 2016; Leitão-Gonçalves et al., 2017; Piper et al., 2017). In *Drosophila melanogaster* for example, the availability of yeast, its main source of protein and AAs, is a key determinant of egg production (Bownes and Blair, 1986; Drummond-Barbosa and Spradling, 2001; Grandison et al., 2009). The fly adapts the rates of egg production to changes in yeast availability drastically and rapidly, within days (Drummond-Barbosa and Spradling, 2001). Ovaries from protein-deprived flies are greatly reduced in size, and their ovarioles contain few or no vitellogenic stage egg chambers (Drummond-Barbosa and Spradling, 2001; Schwartz et al., 1985; Terashima et al., 2005). Stem cells are especially sensitive to the diet. They respond to nutrient availability by modulating their proliferation rates and differentiation (Drummond-Barbosa and Spradling, 2001; Hsu and Drummond-Barbosa, 2009).

Animals have developed two different strategies to adapt food intake to their current internal state. One type of mechanism drives changes in food intake in a feed-forward, anticipatory way, increasing the intake of specific nutrients before the animal is completely deprived of them (Walker et al., 2017). One example of such a mechanism is the increase in salt and yeast intake mediated by the Sex Peptide upon mating in *Drosophila*. During copulation the male-derived Sex Peptide is transferred to the female and binds the neuronal expressed Sex Peptide Receptor SPR leading to an increase in yeast and salt feeding (Ribeiro and Dickson, 2010; Walker et al., 2015). At the circuit level SPR activation leads to the silencing of a dedicated neuronal circuit which

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projects to the central brain to change taste perception and hence the intake of specific nutrients (Corrales-Carvajal et al., 2016; Walker et al., 2015). Importantly, mating does not alter food choice by changing nutrient demand, since yeast and salt appetites are unaltered by the reproductive capacity of the animal (Ribeiro and Dickson, 2010; Walker et al., 2015). Another type of mechanism leads to changes in food intake via feed-back allowing the animal to adapt its behavior to compensate for specific nutrient deficits. As such, flies readily increase yeast intake upon yeast and AA deprivation (Corrales-Carvajal et al., 2016; Ribeiro and Dickson, 2010; Leitão-Gonçalves et al., 2017; Piper et al., 2014, 2017). The neuronal circuits and molecular pathways by which flies homeostatically increase yeast intake to compensate for a lack of AAs are not well understood. One possibility is that similarly to leptin in vertebrates, deficits in specific nutrients are measured in metabolically active tissues which lead to the secretion of a signaling molecule which acts on the brain to alter food intake (Myers et al., 2008). Alternatively, nutrients could be directly sensed by the nervous system to induce adaptive changes in food choice.

In adult female insects, the ovaries are the site of synthesis of multiple signaling molecules, including ecdysone. The hormonally active form of ecdysone, 20-hydroxyecdysone (20E), is produced from a cholesterol precursor derived from dietary yeast ergosterol. In *Drosophila* larvae ecdysteroids are produced in response to prothoracicotropic hormone (PTTH) and nutritional inputs (Colombani et al., 2005; Koyama et al., 2014; Mirth and Shingleton, 2014). The first steps of 20E synthesis occur in the larval prothoracic gland. Pulses of 20E are crucial for the timely development of the animal from the embryonic stage through the three larval instar stages into the adult fly. In the adult the titers of 20E are much lower when compared to earlier developmental stages (reviewed in Schwedes and Carney (2012)). In the adult female, 20E synthesis occurs in the ovaries (Bownes and Blair, 1986; Bownes et al., 1984; Domanitskaya et al., 2014; Hentze et al., 2013; Schwartz et al., 1985). Ecdysone has also been detected in other fly tissues, but it is currently unclear if it gets synthesized in these tissues (reviewed in Galikova et al. (2011), Schwedes and Carney (2012)). In the target tissues, 20E binds to a heterodimeric receptor consisting of EcR and Ultraspiracle, which activates a signaling cascade that alters the expression of target genes (reviewed in Galikova et al. (2011), Schwedes and Carney (2012)). While ecdysone is best known for its role in larval growth (reviewed in Schwedes and Carney (2012)), it is also thought to affect adult physiology regulating, among other things, vitellogenesis and egg production, courtship behavior, adult reproductive diapauses, innate immunity, as well as stress resistance and lifespan (reviewed in Galikova et al. (2011), Schwedes and Carney (2012)). Its function in controlling adult behavior however, still remains poorly explored.

Adult ecdysone levels appear to be responsive to changes in the environment. Titers of ecdysone are elevated in mated females, in males whose courtship advances have been rejected by females and in flies which have been sleep-deprived (reviewed in Schwedes and Carney (2012)). However, while there are reports showing that the availability of dietary yeast alters 20E titers, there is contradictory evidence on how it responds to this stimulus, with some reports suggesting an increase while others, a decrease upon yeast deprivation (Bownes, 1989; Schwartz et al., 1985; Terashima et al., 2005). Intriguingly, ecdysone signaling has been proposed to play an important role in adapting nutrient intake to the reproductive lipid demands of the female (Sieber and Spradling, 2015). Ovarian 20E levels could therefore play an important role in reporting the protein state of the female to the brain to adapt food choice in the adult fly.

In this study we aim at testing: a) if yeast deprivation leads to a change in ovarian ecdysone titers; and b) if ecdysone titers can act on the nervous system of the fly to direct changes in yeast appetite. To do so, we first tested flies that were either kept on a yeast-based medium (YBM) or a diet devoid of yeast for their feeding preference, ovary physiology and ecdysteroid titers. Protein deprived females show a drastic reduction in ovary size and a concomitant increase in yeast consumption compared to fully fed females. Furthermore, we observed a drastic reduction in ecdysone titers in yeast deprived females. To test if there is a causal link between the ecdysone decrease and the alteration of the fly's feeding preference, we used ovo^{D1} mutants which are severely impaired in egg production. Despite the ovaries of these mutant females being very small, these animals also increase yeast consumption after protein deprivation. However, ecdysone titers of these flies are drastically reduced, independently of the nutrient state of the female. Our data are therefore in agreement with ovaries being the prime site of ecdysone production in adults and their physiological state being a major determinant of adult female ecdysone levels. Furthermore, our data indicate that ecdysone is not likely to be involved in controlling food choice upon yeast deprivation, since ovo^{D1} females have constitutively low levels of this hormone without displaying any effect at the level of feeding decisions.

2. Materials and methods

2.1. Drosophila stocks and genetics

For all experiments w^{1118} mated females were used as the control genotype. ovo^{D1} mutant flies used in the experiments were generated by crossing males from the stock ovo^{D1} v^{24}/C(1)DX, y^1 w^1 f^1 (Bloomington #1309) (Oliver et al., 1987) to w^{1118} virgins. The resulting ovo^{D1} v^{24}/w^{1118} females were termed ovo^{D1} mutants throughout the study.

2.2. Drosophila rearing, media, and dietary treatments

Flies were reared on yeast-based medium (YBM) (per liter of water: 8 g agar (NZYTech, PT), 80 g barley malt syrup (Prórida, PT), 22 g sugar beet syrup (Grafschafter, DE), 80 g corn flour (Prórida, PT), 10 g soya flour (A. Centazi, PT), 18 g instant yeast (Saf-instant, Lesaffre), 8 ml propionic acid (Argos), and 12 ml nipagin (Tegospet, Dutscher, UK) (15% in 96% ethanol) supplemented with instant yeast granules on surface (Saf-instant, Lesaffre). To ensure a homogenous density of offspring among experiments, fly cultures were always set with 6 females and 3 males per vial and left to lay eggs for 3 days. 14 days after the culture was started, flies were sorted and transferred to fresh YBM for 2 days and then transferred to fresh YBM for additional 24 h to ensure a well fed state. Subsequently, flies were either kept on YBM for 2 days with a final transfer to fresh food for 24 h (fully fed flies) or transferred to tubes containing paper towels soaked with 5 ml of 100 mM sucrose solution to induce protein deprivation for 3 days. Fly rearing, maintenance, and behavioral testing were performed at 25 °C in climate-controlled chambers at 70% relative humidity in a 12-hr-light-dark cycle (Aralab, FitoClima 60000EH).

2.3. flyPAD feeding assays

flyPAD assays were performed as described in Itskov et al. (2014). For food choice experiments, single flies in different dietary conditions were tested in arenas that contained two kinds of food patches: 10% Yeast and 20 mM Sucrose, each mixed with 1% agarose. Flies were individually transferred to flyPAD arenas by mouth aspiration and allowed to feed for 1 h at 25 °C, 70% relative humidity. The total number of sips per animal over this hour was calculated using previously described flyPAD algorithms (Itskov et al., 2014). Non-eating flies (defined as having fewer than 2 activity bouts during the assay) were excluded from the analysis.

2.4. 20E measurements

Ecdysteroid titers were measured using a protocol adapted from (Mirth et al., 2005; Porcheron et al., 1989a). 50 flies per condition were snap frozen in dry ice and stored at −80 °C awaiting further processing.

Ice cold methanol (1350 µl) was added to each set of flies and samples were homogenized with a pestle. Ecdysteroids were allowed to diffuse from fly tissues overnight at −20°C. Samples were centrifuged in a tabletop centrifuge at top speed (14,000 rpm) and the supernatant recovered into a new eppendorf tube. Methanol was evaporated at room temperature using an Integrated SpeedVac System SPD2010 (Thermo-Savant). Ecdysteroid levels were quantified via competitive Enzyme Immunoassay (EIA) (Cayman Chemicals) using: Precoated (Mouse Anti-Rabbit IgG), EIA 96-Well Solid Plate, 20-Hydroxyecdysone EIA Antiserum, 20-Hydroxyecdysone AChE Tracer, Ellman's Reagent, EIA Buffer and Wash Buffer, following the manufacturer's instructions for processing the samples and measuring 20E concentration. The antiserum detects ecdysone, 20-hydroxyecdysone and other ecdysteroid metabolites including 2-deoxy-20-hydroxyecdysone and 2-deoxyecdysone (Polgar et al., 1996; Porcheron et al., 1976; Porcheron et al., 1989b). The standard curve was generated using 20E serial dilutions (Cayman Chemicals) and results are therefore expressed as 20E equivalents.

3. Results

In order to test the impact of dietary proteins on gonadal physiology and food choice, we manipulated the yeast content of the fly diet; we reared female mated flies in YBM, and sorted them into either YBM or tubes containing 100 mM sucrose (to induce protein deprivation). After three days, the gonads of the females were analyzed. The ovaries of *Drosophila* females are usually composed by 16–20 ovarioles. Each ovariole is comprised of a germarium at the most anterior tip and chains of egg chambers which develop anterior to posterior and culminate in fully developed eggs. The ovaries of fully fed flies show all stages of egg chamber development and contain eggs in the last stage of development (Fig. 1A). As previously reported, we observed that yeast deprivation dramatically changes ovary physiology (Fig. 1B) (Drummond-Barbosa and Spradling, 2001; Schwartz et al., 1985; Terashima et al., 2005). In contrast to fully fed flies, ovaries from protein deprived females are much smaller, containing few or no vitellogenic egg chambers, and ultimately very few or no eggs in the last stages of development (Fig. 1B). In parallel, we also tested these flies for alterations in food choice using the flyPAD technology, which allows for the automatic and quantitative survey of the feeding interactions of single flies with two different nutrient sources (Itskov et al., 2014). In this experiment, we allowed the flies to choose between feeding from 10% yeast or 20 mM sucrose. In agreement with previous reports, we observed that yeast deprived females dramatically increased their feeding from the yeast source compared to females kept on YBM (number of sips) (Fig. 1C) (Corrales-Carvajal et al., 2016; Ribeiro and Dickson, 2010). Yeast deprivation leads to a cessation in egg production and a concomitant increase in yeast appetite.

Because yeast deprivation led to a drastic reduction in ovary size and, in adult flies, ecdysone has been reported to be produced by the female gonads (reviewed in Galikova et al. (2011), Schwedes and Carney (2012)), we tested if ecdysone synthesis was affected by yeast deprivation. We used a colorimetric assay to measure ecdysteroids in fully fed and yeast deprived females. We observed a drastic reduction (3.75X) in the total amount of the hormone in yeast deprived females when compared to fully fed flies (Fig. 2). There is therefore a clear correlation between the protein content of the diet, gonad size, and ecdysone production. These data are fully compatible with a simple model in which protein deprivation causes a decrease in ovary size which is accompanied by a decrease in ecdysone titers.

In adult *Drosophila* females, a high yeast appetite is thought to be mainly necessary for sustaining a high reproductive rate. We therefore hypothesized that gonadal derived ecdysone could serve as a signal to drive the increase in yeast appetite observed upon protein deprivation. To test this hypothesis, we used ovo^{D1} mutants to dissociate the effect of diet on the reproductive system from its effect on behavior. The *Drosophila ovo* gene encodes a putative transcription factor with TFIIIA-like zinc fingers and has been shown to be required for female germline survival and proper oogenesis (Oliver et al., 1987). Three dominant

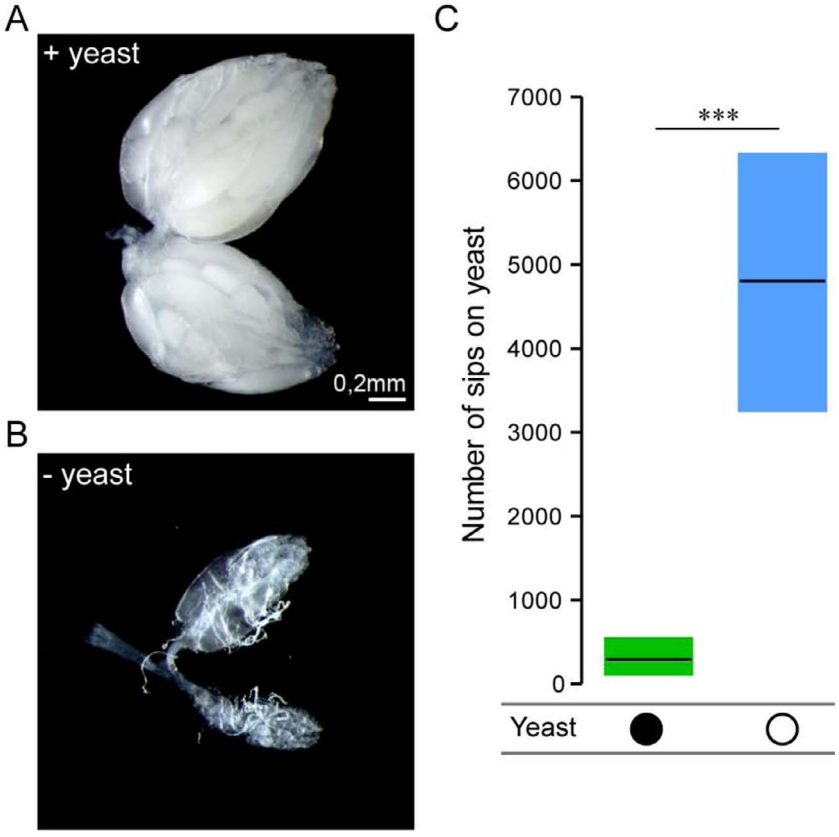

Fig. 1. Protein deprivation impacts flies' gonad physiology and feeding behavior. (A) Ovaries of w^{1118} flies kept on YBM. (B) Ovaries of w^{1118} flies kept on 100 mM sucrose for three days to induce protein deprivation. (C) Number of sips on yeast of flies kept on YBM (represented by filled black circles) or 100 mM sucrose (represented by open black circles). The output parameter from the flyPAD, number of sips, represents the number of feeding interactions of single flies with the food source. Boxes represent upper and lower quartiles with median. Significance was tested using the Mann-Whitney test. n = 42–61. $^{***}p < 0.001$.

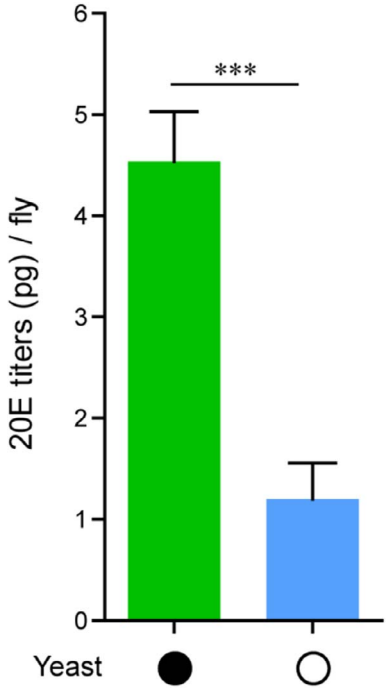

Fig. 2. Ecdysone titers of adult mated flies are modulated by protein availability. w^{1118} females were kept on YBM (represented by filled black circles) or 100 mM sucrose (represented by open black circles) for three days to induce protein deprivation. 20E titers were measured using a colorimetric assay and are here represented as the average and SEM titer per fly. Significance was tested using the Mann-Whitney test. n = 10. $^{***}p < 0.001$.

female-sterile ovo^{D} alleles have been described that cause ovarian abnormalities. They define an allelic series, with ovo^{D1} displaying the strongest phenotype and ovo^{D3} the weakest one. All three ovo^{D} alleles originate from point mutations which create new start codons in the 5′ region of the gene, leading to the addition of extra amino acids at the N terminus of the translated proteins. These alterations lead to a defective protein which interferes with the wild-type protein creating a dominant effect (Mevel-Ninio et al., 1996). ovo^{D1} mutants lack all vitellogenic stage egg chambers, halting oogenesis before vitellogenesis (Oliver et al., 1987). Accordingly, we found ovo^{D1} mutants to have abnormal ovaries with ovarioles containing no vitellogenic egg chambers independently of dietary treatment (Fig. 3A and B). Indeed, the ovaries of these mutants (in fully fed and yeast deprived conditions) look very similar to the ones of protein deprived control flies (Figs. 1B and 3A). We next tested if the mutant flies still showed an increase in yeast appetite upon protein deprivation. In accordance to what has been previously described, these flies behave similarly to the control flies showing a drastic and specific increase in the number of sips on yeast upon deprivation (Fig. 3C; (Ribeiro and Dickson, 2010)). Interestingly, ovo^{D1} mutants showed a general decrease in both yeast and sucrose appetite in fully fed and yeast deprived states when compared to control females (Figs. 3C and S1). These data are in agreement with the idea that the germline has a generalized impact on feeding in *Drosophila*.

Because, ovo^{D1} mutants allow us to dissociate the effects of the protein content of the diet on the germline from its impact on behavioral protein homeostasis, we next asked whether ecdysone amounts in ovo^{D1} mutants reflect the physiology of their gonads or the nutritional state of the animal. We used the same approach as descried above to quantify the 20E levels in fully fed and yeast deprived ovo^{D1} mutants. We found that 20E is present in very low amounts in ovo^{D1} mutants compared to control flies independently of their dietary state (Fig. 2 and 4). The measured levels were similar to the ones found in protein deprived control flies with fully fed ovo^{D1} females containing 3.4X less 20E than the control animals (Fig. 4). Since fully fed ovo^{D1} mutants do not show higher yeast appetite when compared to control fully fed animals, we can conclude that it is unlikely that a decrease in 20E is at the basis of the alterations in feeding observed in protein deprived flies. Furthermore, given that the detected levels of 20E in yeast deprived mutant and control females are the same, changes in ecdysone signaling might also not explain the reduction in yeast feeding observed in ovo^{D1} females. Overall, our data do not support the idea that gonadal derived ecdysone or other signals are at the basis of the increase in yeast appetite observed upon protein deprivation.

4. Discussion

It is well established that nutrition has a profound impact on the reproductive system of insects. In particular, the amount of amino acids and protein ingested has been positively correlated with the increase in reproductive outcome (Drummond-Barbosa and Spradling, 2001;

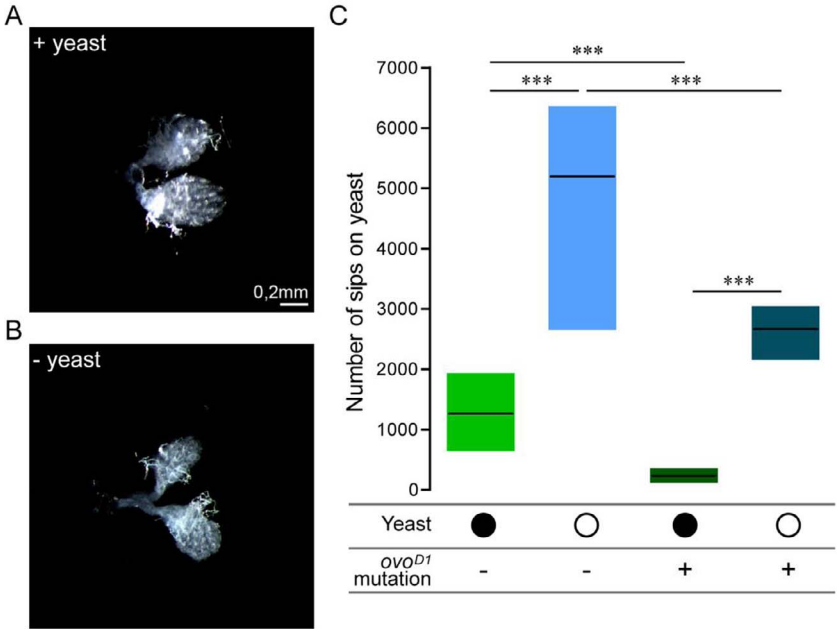

Fig. 3. ovo^{D1} germline mutants contain protein deprived-like ovaries but feeding choices are not affected. (A) Ovaries of ovo^{D1} mutant females kept on YBM. (B) Ovaries of ovo^{D1} mutant females kept on 100 mM sucrose for three days to induce protein deprivation. (C) Number of sips on yeast of flies kept on YBM (represented by filled black circles) or 100 mM sucrose (represented by open black circles). Filled black circles represent the presence of yeast. The – sign represents the absence while the + sign represents the presence of the ovo^{D1} mutation. Boxes represent upper and lower quartiles with median. Significance was tested using the Mann-Whitney test with Bonferroni correction. n = 41–48. $^{***}p < 0.001$.

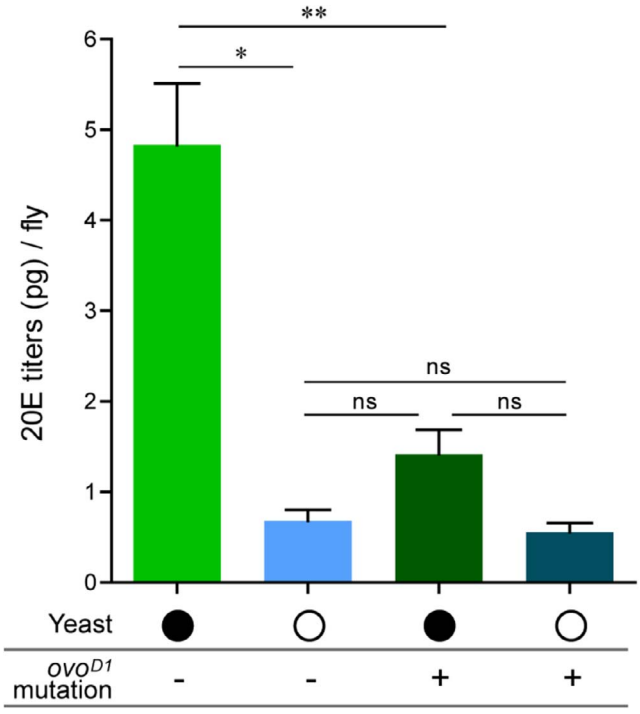

Fig. 4. Ecdysone titers in adult ovo^{D1} females are decreased to levels similar to the levels observed in yeast deprived mated controls. w^{1118} or ovo^{D1} females were kept on either YBM or 100 mM sucrose for three days to induce protein deprivation. 20E titers were measured using a colorimetric assay and are represented as the average and SEM titer per fly. Filled black circles represent the presence of yeast. The – sign represents the absence while the + sign represents the presence of the ovo^{D1} mutation. Significance was tested using the Mann-Whitney test with Bonferroni correction. n = 4–7. $^{**}p < 0.01$, $^{*}p < 0.05$, ns $p \geq 0.05$.

Hansen et al., 2004; Hosios et al., 2016; Leitão-Gonçalves et al., 2017; Piper et al., 2014, 2017). The female gonads are the site of synthesis of several hormones that act systemically and impact the animal physiology such as insulin like peptides and ecdysone (Garelli et al., 2012; Schwartz et al., 1989; Terashima et al., 2005). In this study, we focused on testing how nutrition affects the production of ecdysone and what could be the potential influence of this hormone on food choice behavior. We find that, while protein deprivation dramatically reduces the reproductive capacity of the animal and decreases ecdysone titers, these two events are not causally related to the increase in yeast feeding observed upon protein deprivation. Ecdysone signaling and the physiology of the reproductive system can therefore be functionally separated from food choice behavior.

Protein deprivation leads to a dramatic reduction in egg production and in the size of the female gonads. Since in the adult female, the major site for ecdysone synthesis is the gonads, it is not surprising that we observe a dramatic reduction in this hormone in protein deprived females as well as in females with impaired oogenesis (Fig. 2). This finding is not due to unspecific effects of the ovo^{D1} mutation as germless *tudor* mutants also display a decrease in total ecdysone levels when compared to control females (Parisi et al., 2010). While earlier studies had also suggested that starvation affects 20E titers in adult females (Schwartz et al., 1985), these results stand in contrast to other report suggesting that 20E is increased in protein deprived flies (Terashima et al., 2005). Our measurements support the view that protein deprivation leads to a drastic decrease of 20E in adult mated females. The observation that mated ovo^{D1} females, with their rudimentary ovaries, also show a strong reduction in 20E titers further substantiates this interpretation. The reported discrepancies in the impact of nutrition on 20E titers across different studies may be explained by differences in the mating state of the females or the measurement method. Whatever the difference our results support the parsimonious view that protein deprivation acts on the mated female gonad to reduce 20E titers.

Drosophila melanogaster is able to modulate its feeding behavior in order to prioritize the intake of specific nutrients. If flies are protein deprived, they will increase the consumption of proteinaceous yeast (Fig. 1). Since dietary proteins play a key role in sustaining high reproductive levels, we tested if the observed reduction in ecdysone could lead to a change in the homeostatic yeast appetite observed upon protein deprivation. To ask this question we used *ovo* mutants which have been extensively studied for its role in oogenesis progression (Oliver et al., 1987). We used ovo^{D1} mutants which halt oogenesis at stage 4, before the onset of vitellogenesis. Given that ovo^{D1} mutants show a constant low level of ecdysone but are still able to increase yeast feeding upon protein deprivation, we conclude that ecdysone is not the instructive signal controlling protein appetite. Importantly, ovo^{D1} mutants have a slight extension of lifespan (Clancy et al., 2001; Sgro and Partridge, 1999) and to display normal metabolic gut remodeling upon mating (Reiff et al., 2015) suggesting that they are not physiologically impaired.

Our finding that ecdysone is not the instructive signal controlling yeast appetite has implications beyond *Drosophila,* as dietary AAs affect both gonadal physiology and feeding behavior in many insects, including mosquitoes. Experiments in mosquitoes have led to contested results regarding a potential involvement of ecdysone in controlling host-seeking behavior (Beach, 1979; Klowden, 1980; Beach, 1980). Our findings, support the conclusion that ecdysone is unlikely to be a critical factor modulating blood feeding in mosquitoes (Klowden, 1981).

It is interesting to note that ovo^{D1} females show an overall reduction in feeding behavior, independent of their nutritional status and the nutrient on which they are feeding. Sieber and Spradling have proposed that the germline upregulates ecdysone synthesis to induce an increase in food intake (Sieber and Spradling, 2015). This increase in food intake is supposed to support the accumulation of lipids and glycogen stores required for proper oocyte maturation. Our observation that flies without a germline show an overall decrease in feeding supports their model that the germline plays an important role in bulk food intake. In addition, our results clearly suggest that the regulation of overall food intake and the induction of nutrient specific appetites are phenotypically separable, supporting the view that the animal is able to regulate overall feeding rate and food choice independently.

The emerging picture is that in *Drosophila melanogaster* yeast appetite is independently regulated by both feed forward anticipatory mechanisms and feedback homeostatic mechanisms: mating induces a feed-forward increase in yeast appetite and salt appetites (Ribeiro and Dickson, 2010; Walker et al., 2015) to preemptively alter food choice towards food sources supporting a high level of reproduction (Walker et al., 2017). Changes in food preference induced by mating, rely on changes in gustatory processing mediated by a canonical postmating circuitry transmitting the mating information to the central brain (Walker et al., 2015). These behavioral alterations are further supported by the anticipatory metabolic remodeling of organs, such as the intestine (Reiff et al., 2015). Importantly, these alterations seem to be independent of the actual use of nutrients by the reproductive system as flies with impaired oogenesis do not show alterations in these anticipatory mechanisms. These feed-forward anticipatory mechanisms are then complemented by feedback homeostatic mechanisms which inform the animal of the lack of specific nutrients such as AAs, allowing it to adapt feeding decisions to unpredictable changes in nutrient availability (Walker et al., 2017). Gonads can indeed sense the availability of dietary proteins and AAs (Bownes and Blair, 1986; Drummond-Barbosa and Spradling, 2001; Grandison et al., 2009; Piper et al., 2014, 2017) and adapt stem cell proliferation and oogenesis to the nutrient level of the animal. Given the importance of dietary proteins for reproduction, one could therefore expect gonadal ecdysone to be a signal informing the brain of the nutrient state of the animal. Our experiments, however, suggest that the gonadal changes induced by the lack of dietary proteins do not impinge on food choice behavior via ecdysone. The increase in

yeast appetite upon dietary protein deprivation is therefore caused by nutrient sensing in other organs. Previous results suggest that the nervous system could directly sense the lack of AAs to induce the appetite for proteinaceous foods in insects (Ribeiro and Dickson, 2010; Simpson and Simpson, 1992). Clarifying the site of AA sensing which lead to the induction of protein appetites in insects is therefore an important avenue of research for understanding insect nutrient homeostasis.

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Author contributions

Conceived and developed the project: ZCS, CR; Performed experiments: ZCS; Performed data analysis and interpretation: ZCS, CR; Wrote the manuscript: ZCS, CR.

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.2017.08.006.

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