1
2

3

4

5

6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

What happens after a blood meal? A transcriptome response from the main tissues involved in egg production in *Rhodnius prolixus*, an insect vector of Chagas disease

Short title: Transcriptome analysis on *Rhodnius prolixus*, a vector of Chagas disease

Jimena Leyria^{1*}, Ian Orchard¹ and Angela B. Lange¹

¹Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada

* Corresponding author

Email: jimena.leyria@utoronto.ca (JL)

5
6

28Abstract

29

30 The blood-sucking hemipteran *Rhodnius prolixus* is a vector of Chagas disease, one of
31the most neglected tropical diseases affecting several million people, mostly in Latin America.
32The blood meal is an event with a high epidemiological impact since in adult mated females it
33initiates the production of hundreds of eggs. By means of RNA-Sequencing (RNA-Seq) we have
34examined how a blood meal influences mRNA expression in the central nervous system (CNS),
35fat body and ovaries in order to promote egg production, focusing on tissue-specific responses
36under controlled nutritional conditions. We illustrate the cross talk between reproduction and a)
37lipids, proteins and trehalose metabolism, b) neuropeptide and neurohormonal signaling, and c)
38the immune system. Overall, our molecular evaluation confirms and supports previous studies
39and provides an invaluable molecular resource for future investigations on different tissues
40involved in successful reproductive events. Analyses like this can be used to increase the chances
41of developing novel strategies of vector population control by translational research, with less
42impact on the environment and more specificity for a particular organism.

43

44Author summary

45

46 The blood-sucking hemipteran *Rhodnius prolixus* is one of the main vectors of Chagas
47disease. The blood meal is an event with a high epidemiological impact since in adult mated
48females, blood-gorging leads to the production of hundreds of eggs. This work describes an in-
49depth central nervous system (CNS), ovary and fat body transcriptome analysis, focusing on
50transcripts related to blood intake which may be relevant in promoting egg production. To date,

7
8

9

10

51the principle focus in Chagas disease prevention is on the elimination of triatomine vectors and
52their progeny. This work will serve as a starting point for initiating novel investigations on
53targets identified with a potential for use in vector control; for example using specific genes to
54generated symbiont-mediated RNAi, a powerful technology which provides a novel means in
55biocontrol against tropical disease vectors.

56

57

58**Key words:** Insect, Triatominae; transcriptome; reproduction; lipid metabolism, trehalose
59metabolism, neuropeptides, immune regulation.

60

61

62

63

64

65

66

67

68

69

70

11

12

13

14

71Introduction

72

73 Insects, which represent more than half of all living organisms on earth, have a close
74relationship with human beings. To many of them, we can ascribe a negative interaction, for
75example those that act as carriers of disease. Chagas disease, one of the most neglected tropical
76diseases, is caused by the protozoan *Trypanosoma cruzi*, which is transmitted to mammalian
77hosts primarily by blood-feeding insects, the triatomines [1]. This disease affects 6-7 million
78people, mostly in Latin America, but because of migration the disease has spread to other
79continents [2]. To date, treatment of the chronic phase of this disease is limited [3], resulting in
802000 deaths per year [1], although it is known that Chagas disease is an under-reported cause of
81death [4]. The principle focus in Chagas disease prevention is on the elimination of triatomine
82vectors from human homes. Currently, the most heavily used option is chemical control,
83although resistance to these insecticides has been reported in the last decade [5]. Furthermore,
84the devastating impact of chemical insecticides on the environment and other organisms, such as
85beneficial insects, can no longer be ignored [6].

86 Triatomines have developed an integrated control over the reproductive system, whereby
87different tissues work with extreme precision and coordination to achieve successful production
88of progeny. There are three tissues that work in concert to promote reproduction; the central
89nervous system (CNS), fat body and ovaries. The CNS contains neuroendocrine cells that
90synthesize neuropeptides involved in the coordination of events that promote egg production.
91These neuropeptides are produced as large precursors, which are then cleaved and modified to
92become biologically active neuropeptides [7]. These neuropeptides are secreted as
93neuromodulators or neurohormones to act via specific receptors [8]. With regard to reproduction,

15

16

17

18

94these receptors are located on the fat body and ovaries. The fat body is a multifunctional organ
95analogous to vertebrate adipose tissue and liver. It is considered an interchanging center,
96remotely integrating with the CNS to regulate physiology by sensing hormonal and nutritional
97signals and responding by mobilizing stored nutrients such as proteins, carbohydrates and lipids,
98for use in egg formation, or during periods of inactivity or nutritional shortage [9-10]. Apart
99from these storage functions, the fat body is also involved in the regulation of hematopoiesis,
100innate immune homeostasis and detoxification [10].

101 In oviparous organisms, including triatomines, embryonic development occurs apart from
102the maternal body. Egg survival, therefore, depends on the utilization of previously stored
103material taken up by the oocytes, such as proteins, lipids, carbohydrates and other minor
104components, all of which are synthesized mainly by the fat body [11]. Insect oocytes are specific
105structures designed to select, internalize, and store nutrients, such as yolk granules and lipid
106droplets. The process of yolk deposition is termed vitellogenesis, which represents a phase of
107accelerated egg growth leading to the production of mature eggs in a relatively short period of
108time [11]. The CNS-fat body-ovary axis is essential for triatomines to produce viable eggs.
109Interestingly, the trigger for this interaction is a single blood meal. Although in some colonies of
110the triatomine, *Rhodnius prolixus*, unfed females can make a small number of eggs from
111resources that may remain after moulting to an adult (autogeny) [12], the large batch of eggs is
112triggered by ingestion of a blood meal. After a blood meal, a *R. prolixus* female can produce
11330–50 eggs during the following three weeks [13]. For this reason, knowledge of the molecular
114and cellular mechanisms used in egg formation are essential to develop novel strategies of vector
115population control.

19

20

21
22

116 In addition to being a main vector of Chagas disease, with high epidemiological
117relevance for easily colonizing domestic habitats [14], *R. prolixus* has been the subject of intense
118investigations over the past century, which have contributed to our understanding of important
119aspects of metabolism and physiology in insects [15]. It is important to highlight that the
120complete genome of *R. prolixus* has been published [16] and, therefore, many new questions can
121be asked and answered with regard to insect physiology/endocrinology. Next-generation
122sequencing allows us to study biological systems at the genomic level to link mRNA sequences
123with specific biological functions of specific tissues during a particular stage or state. Recently,
124by transcriptome analysis we reported an up-regulation of transcripts involved in insulin-like
125peptide/target of rapamycin (ILP/ToR) signaling in unfed insects. However, we demonstrated
126that this signaling pathway is only activated in the fat body and ovaries of fed insects. Thus, we
127demonstrated that unfed females are in a sensitized state to respond to an increase of ILP levels
128by rapidly activating ILP/ToR signaling after a blood meal [17]. Here, we examine how a blood
129meal influences CNS, fat body and ovary gene expression to promote egg production; focusing
130on details associated with tissue-specific responses in particular nutritional states. Our data opens
131up avenues of translational research that could generate novel strategies of vector population
132control, with less impact in the environment and with more specificity for a particular organism,
133such as using specific genes to generated symbiont-mediated RNAi; a powerful technology
134which provides a potential means in biocontrol against tropical disease vectors [18].

135

136**Materials and Methods**

137

138**Insects**

23
24

25

26

139 Insects were maintain in incubators at 25°C under high humidity (~50%). Newly-
140emerged adult females (10 days post-ecdysis) were segregated, and placed together with a
141recently fed male to copulate. Then, female insects were fed on defibrinated rabbit blood
142(Cedarlane, Burlington, CA) to initiate egg growth. Only insects that fed 2.5 to 3 times their
143initial body weight were used for the experiments. CNS, fat body (FB) and ovaries (OV) from
144adult mated females were dissected at 10 days post-ecdysis for the unfed condition (UFC) and 3
145days post-feeding as the fed condition (FC), according to Leyria et al. [17]. Insects in the fed
146condition will have begun vitellogenesis and egg growth.

147

148**Transcriptomic data analysis**

149 Read sequences were obtained from Leyria et al. [17]. This study reported transcriptomes
150of CNS, FB and OV from fed and unfed females. The raw sequence dataset of this project is
151registered with the National Center for Biotechnology Information (NCBI) under PRJNA624187
152and PRJNA624904 bioprojects. A detailed description of our bioinformatic pipeline can be
153found in Leyria et al. [17]. Briefly, CNS, OV and ventral and dorsal FB of *R. prolixus* females
154were dissected in cold autoclaved phosphate buffered saline (PBS, 6.6 mM Na₂HPO₄/KH₂PO₄,
155150 mM NaCl, pH 7.4). Three independent experiments were analyzed (n = 3) with each n
156composed of a pool of 10 tissues. RNA extraction was performed with Trizol reagent (Invitrogen
157by Thermo Fisher Scientific, MA, USA), followed by DNase treatment (Millipore-Sigma, WI,
158USA) and then repurified with PureLink RNA Mini Kit (Ambion by Thermo Fisher Scientific,
159MA, USA). Libraries for sequencing were made from high quality RNA that were generated
160using NEBNext Ultra RNA Library Prep Kit for Illumina (New England Biolabs, MA, USA)
161following manufacturer's recommendations. The libraries were sequenced on Illumina HiSeq

27

28

29

30

162platforms (*HiSeq 2500*) at the Novogene sequencing facility (California, USA). Raw data were
163recorded in a FASTQ file, which contains sequence (reads) and corresponding sequencing
164quality information. Fastq format were first processed through in-house perl scripts, where clean
165data (clean reads) were obtained by removing reads containing the adapter, reads containing
166poly-N and low quality reads from raw data. Also, Q20, Q30 and GC content from the clean data
167were calculated. All the downstream analyses were based on the clean data [17].

168

169Differential expression analysis

170 First, clean reads were aligned to the reference genome using HISAT2 software. After
171that, HTSeq v0.6.1 was used to count the number of reads mapped to each gene. FPKM
172(expected number of Fragments Per Kilobase of transcript sequence per Millions base pairs
173sequenced) of each gene were calculated based on the length of the gene and number of reads
174mapped to the gene. In general, an FPKM value of 0.1 was set as the threshold for determining
175whether the gene is expressed or not. Differential expression analysis of two nutritional
176conditions were performed using the DESeq R package (1.18.0). DESeq provides statistical
177routines for determining differential expression in digital gene expression data using a model
178based on the negative binomial distribution. The resulting *P*-values were adjusted using the
179Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an
180adjusted *P*-value < 0.05 found by DESeq were assigned as differentially expressed. Gene
181Ontology (GO) enrichment analysis of differentially expressed genes was implemented by the
182Goseq R package, in which gene length bias was corrected. GO terms with corrected *P*-value
183less than 0.05 were considered significantly enriched by differential expressed genes.

31

32

33
34
184

185 Validation of quantitative gene expression

186 To validate Illumina sequencing results, 7 genes were chosen at random and their
187 expressions were analyzed by quantitative polymerase-chain reaction (RT-qPCR) [17]. Briefly
188 total RNA was extracted as described above, followed by cDNA synthesis using the High
189 Capacity cDNA Reverse Transcription Kit (Applied-Biosystems, by Fisher Scientific, ON,
190 Canada). RT-qPCR was performed using an advanced master mix with super green low rox
191 reagent (WISENT Bioproducts Inc, QC, Canada). Three independent experiment were
192 performed (n=3) with each n composed of a pool of 5 tissues. Each reaction contained 3
193 technical replicates and were carried out using a CFX384 Touch™ Real-Time PCR Detection
194 System (BioRad Laboratories Ltd., Mississauga, ON, Canada). The primers used (by Sigma-
195 Aldrich, ON, Canada) for amplification are shown in S1 Table. Quantitative validation was
196 analyzed by the $2^{-\Delta\Delta C_t}$ method. All reactions showed an amplification efficiency higher than 95
197%. β -actin, which was previously validated for transcript expression in FB and OV from *R.*
198 *prolixus* at different nutritional conditions [17], was used as the reference gene. For each pair of
199 primers a dissociation curve with a single peak was seen, indicating that a single cDNA product
200 was amplified. Specific target amplification was confirmed by automatic sequencing (Macrogen,
201 NY, USA). The correlation coefficient between Illumina RNA sequencing and RT-qPCR data
202 was analyzed by the Pearson test.

203

204 Lipid and carbohydrate measurements

35
36

37

38

205 Ovaries and ventral and dorsal FB were dissected from insects during UFC and FC under
206cold *R. prolixus* saline (NaCl 150 mM, KCl 8.6 mM, CaCl₂ 2.0 mM, MgCl₂ 8.5 mM, NaHCO₃
2074.0 mM, HEPES 5.0 mM, pH 7.0). Total lipids and carbohydrates from tissues were measured by
208colorimetric assays as previously described [19]. Briefly, the tissues were placed in either 500 µl
209of isopropanol (for lipid quantification) or 500 µl 10 % cold trichloroacetic acid (TCA, for
210carbohydrate quantification), homogenized and then centrifuged for 10 min at 20 °C and 8000 g.
211For lipid quantification, 400 µl of the supernatants were transferred to 1.5 ml tubes containing
212100 µL of 1 M KOH. Then, the tubes were incubated at 60°C for 10 min and once they were
213cool, 100 µl of sodium periodate solution (11.6 mM sodium periodate in 2 N glacial acetic acid)
214was added. After 10 min of incubation at room temperature, 600 µl of chromogenic solution (40
215ml of 2 M ammonium acetate, 40 ml isopropanol, 150 ml acetyl acetone) were added to the
216samples and incubated for 30 min at 60 °C. The resultant colour was measured at 410 nm using a
217plate reader spectrophotometer (Cytation 3 Imaging Reader, BioTek, Winooski, VT, USA). A
218standard curve of triglycerides ranging from 0 to 60 µg was run independently and in parallel
219with the experimental samples. FB and OV carbohydrate content was measured using the
220anthrone colorimetric assay. Briefly, 50 µl of the supernatants after TCA precipitation were
221mixed with 500 µl of anthrone solution (26 mM anthrone, 1.31 mM thiourea, 66 % sulfuric acid)
222and incubated for 20 min at 100 °C. The samples were allowed to cool in the dark for 15 min and
223then quantified at 620 nm using a plate reader spectrophotometer described. A standard curve
224was run in parallel with the experimental samples using a 0 - 40 µg range of trehalose. Proteins
225were measured according to Bradford [20] processing the tissue as described previously [17].
226Three independent experiments were analyzed (n=3) for each measurement with each n
227composed of a pool of 5 tissues.

39

40

10

41

42

228

229Results and Discussion

230 We were surprised to observe no major gene differences in the CNS between the UFC
231and FC. None of the GO functional terms were enriched in the CNS under these different
232nutritional states. We chose 3 days post-blood meal as the fed condition because of the
233morphological changes observed in the FB and OV (Leyria et al., 2020). The days chosen to
234monitor transcriptional regulation are appropriate for FB and OV but apparently not for CNS.
235Possibly for the CNS, transcriptional regulation begins early after a blood meal to control
236remotely molecular, biochemical and physiological changes that we then observed in the FB and
237OV during the FC. Using *R. prolixus* adult insects, Sterkel et al. [21] reported a quantitative
238proteomic analysis of the post-feeding response from CNS in 3 different conditions: unfed, 4 h
239and 24 h after blood intake. These researchers found only 4 neuropeptides (NVP-like, ITG-like,
240kinin-precursor peptide and neuropeptide-like precursor 1 (NPLP1)) that were significantly up-
241regulated in response to the blood meal. Taken together, this appears to indicate that the changes
242in the transcriptional and protein levels in the CNS of *R. prolixus* adult insects occur quickly or
243more slowly so that it is difficult to find any changes. For this reason, below, we focused our
244attention on the FB and OV and reflect on the CNS transcriptome analysis when making
245reference to peptide/hormone signaling.

246 To validate Illumina sequencing, 7 mRNAs were chosen and their relative transcript
247abundance in FB and OV in both nutritional states monitored by RT-qPCR. A good correlation
248was found between RNAseq and RT-qPCR data; the Pearson tests were 0.9311 (to FB) and
2490.9109 (to OV), with a statistical significance of $p < 0.01$. Multiples genes from these
250transcriptomes were also validated using RT-qPCR by Leyria et al. [17].

43

44

45

46

251

252GO enrichment analysis

253 Nutrients are essential for energy homeostasis of any organism and important changes in
254nutrient stores occur between feeding and non-feeding periods and also more remarkably in adult
255insects during reproductive processes [9, 22]. GO enrichment was used to assign a functional
256classification to differentially expressed genes (DEGs). All DEGs categorize into two main
257groups: cellular components and biological processes. In cellular components, they are divided
258into 21 terms which are significantly up-regulated in FB_FC with respect to FB_UFC (Fig 1A).
259The most represented cellular components terms are cell parts involved in protein synthesis, as is
260to be expected since the FB is the main synthesis and secretory organ responsible for the
261production of virtually all haemolymph proteins. With regard to biological processes, the main
262terms in the FB are involved in biosynthesis and lipid and carbohydrate metabolism (Fig 1B).
263Recently, by examining KEGG enrichment we reported that the “ABC transporters pathway”,
264transporters which use energy to translocate substrates (e.g., sugar, lipid and peptides) across cell
265membranes, is up-regulated in FB_FC, which shows that the synthesized nutrients are released,
266in this case likely to support vitellogenesis [17]. In the OV, the main terms of cellular
267components and biological processes which are significantly up-regulated in OV_FC with
268respect to OV_UFC are related to lipid, carbohydrate and protein metabolism, insect hormone
269biosynthesis, and yolk granule formation (specialized structures which stores all nutrients which
270are used as substrates for embryogenesis and maintenance of the newly hatched nymph) (Fig 2A
271and B). These nutrients are mostly proteins, lipids and carbohydrates, produced by the FB,
272released into the hemolymph and subsequently taken up by the oocytes [23]. As we anticipated
273in light of the results of the GO enrichment, lipid, protein and carbohydrate levels in the FB and

47

48

49
50

274OV are increased in fed females (Fig 3A and B), as reported in other vectors of Chagas' disease
275[22, 24-25]. In addition, it is clear that stored proteins are always the major component in both
276tissues, followed by lipids and then carbohydrate stores.

277

278**Fig 1. Distribution of differentially expressed genes (DEG) in the fat body annotated by GO**
279**enrichment analysis, associated with cellular components and biological processes.** The GO
280enrichment bar chart presents the number of DEG enriched in cellular component (A) and
281biological process (B). The y-axis is GO terms enriched and the x-axis is the number of DEG.
282GO terms with corrected *P*-value less than 0.05 were considered significantly enriched in DEG
283(comparing FB_FC vs FB_UFC). The most significant enriched terms were selected. FB_FC, fat
284body in fed condition; FB_UFC, fat body in unfed condition.

285

286**Fig 2. Distribution of differentially expressed genes (DEG) in ovaries annotated by GO**
287**enrichment analysis, associated with cellular components and biological processes.** The GO
288enrichment bar chart presents the number of DEG enriched in cellular component (A) and
289biological process (B). The y-axis is GO terms enriched and the x-axis is the number of DEG.
290GO terms with corrected *P*-value less than 0.05 were considered significantly enriched in DEG
291(comparing OV_FC vs OV_UFC). The most significant enriched terms were selected. OV_FC,
292ovary in fed condition; OV_UFC, ovary in unfed condition.

293

294**Fig 3. Protein, lipid and carbohydrate content in the fat body and ovaries of females during**
295**the unfed (UFC) and fed condition (FC).** Fat body and ovaries were dissected from adult
296females during the unfed and fed condition. The tissues were homogenized and nutrient extracted

51
52

53

54

297and quantified as described in Materials and Methods. The results for total lipid, carbohydrate
298and protein content were graphed as the mean $\pm$ Standard Error of the Mean (SEM) from three
299independent experiments. Graphs and statistical tests were performed using GraphPad Prism 7
300(GraphPad Software, CA, USA, www.graphpad.com). All datasets passed normality and
301homoscedasticity tests. The statistical significance of the data was calculated using Student's t-
302test. A P value < 0.05 was considered statistically significant.

303

304Protein and hormone analysis

305 Vitellogenins (Vgs), the main yolk protein precursors (YPPs), are large molecules
306synthesized predominantly by the FB, secreted into the hemolymph and then transported to the
307OVs. The number of genes encoding insect Vgs varies from one to several depending on the
308species [26]. Our results show that the mRNA levels for Vgs are considerable higher in the FB
309with respect to the OV, which is not surprising (Fig 4A). In the FB transcripts for *Vg1* and *Vg2*
310are up-regulated during vitellogenesis (FB_FC), with *Vg1* having the highest expression (Fig 4A
311and S2 Table). In *Triatoma infestans*, a triatomine related to *R. prolixus*, *Vg1* and *Vg2* genes are
312expressed at relatively low levels during the UFC and both Vg transcripts are up-regulated after
313blood-feeding [27]. Recently, by KEGG enrichment we reported “amino sugar and nucleotide
314sugar metabolism” and “N-Glycan biosynthesis” are pathways up-regulated in the FB of fed
315females [17]. Glycosylation is a critical post-translational modification to obtain the proper
316protein structure for adequate protein function and for Vgs glycosylation is a step necessary for
317folding, processing and transport to the oocyte [28]. As previously reported in *R. prolixus* [29],
318our results suggest that Vg synthesis also occurs in the OV, with *Vg* transcripts up-regulated after
319a blood meal and *Vg1* levels higher than *Vg2* (Fig 4A and S2 Table). Interestingly, in *T. infestans*

55

56

57

58

the *Vg2* transcript is quantitatively more important than *Vg1* in OV after feeding [27]. The
vitellogenin receptor (VgR) mRNA expression, the endocytic receptor responsible for Vg uptake
by oocytes, is up-regulated in OV from unfed insects (Fig 4A and S2 Table), contrary to
expectation since Vg uptake occurs after a blood meal. However, as expected the main KEGG
enrichment pathways involved in receptor-mediated endocytosis signaling (endocytosis,
lysosome and phagosome pathways) are enriched in OV_FC of *R. prolixus* [17]. This result
indicates that even when the OV expresses high endocytic receptor transcript levels in the UFC,
only after a blood meal does the endocytic process occur. Similarly, in cockroaches VgR mRNA
levels remain low during the vitellogenic phase [30-31]. A similar pattern of high VgR mRNA
levels in non-reproductive stages and low levels during vitellogenesis is found not only in insects
but also in oviparous vertebrates [32-33]. It can be interpreted as a recycling of VgR protein during
the vitellogenic period. In contrast to these observations, in the mosquito *A. aegypti*, VgR
mRNA starts to rise one day after the adult moult, continues to increase dramatically during the
vitellogenic period, and then peaks one day after the blood meal [34]. On the other hand, using
R. prolixus females, Oliveira et al. [35] described another YPP, a 15-kDa protein called
Rhodnius heme binding protein (RHBP), which works as an antioxidant agent in hemolymph.
After the blood meal, a large amount of heme is released from hemoglobin, crosses the digestive
barrier and reaches the hemolymph, where it is sequestered by RHBP. Here, we show that in the
FB, *RHBP* mRNA levels are up-regulated in females 3 days after feeding (Fig 4A and S2 Table).
The increase of synthesis of YPPs in FB_FC coincides with the KEGG analysis reported
recently, where we show an enrichment of “biosynthesis of amino acids pathway” [17].

The Wnt signaling pathway was first discovered as a key event in *D. melanogaster*
development [36]. The Wnt (glycoprotein ligand) and Frizzled (Fz, transmembrane Wnt

59

60

15

61

62

343receptor) proteins interact with structural components at the cell surface to initiate the signaling
344cascades that result in transcriptional regulation of gene expression. In *A. aegypti*, a fundamental
345role of Frizzled 2 (Fz2) was reported in egg production [37]. Here, we find that *Wnt* and *Fz2*
346mRNA levels are up-regulated in OV_FC (Fig 4A and S2 Table). Additionally, Wnt and ToR
347signaling interact synergistically in the vitellogenic process [37] and supporting this finding, we
348showed mTOR signaling is active in OV_FC [17]. Also, the non-canonical Wnt pathway
349indicates that Wnt/Fz signaling leads to the release of intracellular calcium through trimeric G
350proteins [37]. The calcium release and intracellular accumulation activates several Ca^{2+} - sensitive
351proteins, including protein kinase C (PKC), calcineurin and calcium/calmodulin-dependent
352kinase II (CamKII). In *A. aegypti* it was found that juvenile hormone (JH) activates the
353phospholipase C (PLC) pathway and quickly increases the levels of Ca^{2+} for the activation and
354autophosphorylation of CaMKII, which is involved in patency development [38]. On the other
355hand, in many animals, a rise in intracellular Ca^{2+} levels is the trigger for egg activation, the
356process by which an arrested mature oocyte transitions to prepare for embryogenesis. Genetic
357studies have uncovered essential roles for the calcium-dependent enzyme calcineurin in
358*Drosophila* egg activation [39]. By DEG analysis, we demonstrate an up-regulation of *PKC* and
359*calcineurin* in OV from fed insects (Fig 4A and S2 Table). In *R. prolixus*, earlier studies by
360Ilenchuk et al. [40] suggested that a PKC might be involved in patency and Vg uptake but until
361now the receptors or molecular mechanisms responsible for this cascade are unknown. The
362results we observe in vitellogenic oocytes of *R. prolixus* could be indicative of a relationship
363between patency and Wnt/Fz2/ Ca^{2+} signaling. Methoprene-tolerant (Met), which encodes a
364transcription factor of the bHLH-PAS family, was reported to be a JH receptor [41]. *Krüppel*
365*homolog 1* (Kr-h1), identified as the main JH primary-response gene activated by Met [41], is

63

64

65
66

366up-regulated in OV_FC (Fig 4A and S2 Table), which supports the hypothesis that in *R. prolixus*,
367JH is working directly on OVs to stimulate egg formation.

368

369**Fig 4. Heat map comparing the mRNA expression levels of proteins related to reproduction**
370**(A) and Notch signaling pathway (B) in fat body and ovaries of females in different**
371**nutritional conditions.** The input data is the readcount value from the gene expression level
372analysis after normalization and is presented by means of a colour scale, in which
373green/yellow/red represent lowest/moderate/highest expression. DESeq was used to perform the
374analysis.

375

376 Heat shock proteins represent different protein families based on their sequence
377homology and molecular masses. Among them, Heat shock protein 70 family (Hsp70) is highly
378conserved. The expression of Hsp70 is considered a good marker for the inducible stress
379response in an organism [42]. In *T. infestans* Hsp70 is strongly expressed in unfed insects [43].
380Similarly, in *R. prolixus*, we find that *Hsp70* is up-regulated in the FB from unfed females (Fig
3814A and S2 Table), a condition inherently associated with a stressful situation. Glucose-regulated
382protein of 78 kDa (Grp78) is a member of the Hsp70 family which acts as a chaperone to
383facilitate protein folding and to inhibit protein aggregation of new peptides. Interestingly, in
384*Locusta migratoria*, Grp78 was reported as a regulatory factor of Vg synthesis and cell
385homeostasis in the FB via JH signaling [44]. In *R. prolixus*, we show a significant up-regulation
386of Grp78-like protein in both FB and OV from fed insects (Fig 4A and S2 Table). This result
387suggests a novel regulatory mechanism involved in the vitellogenic process of *R. prolixus*.

67
68

69
70

388 Notch is a receptor that directly translates information of cell-cell contact to gene
389expression in the nucleus [45]. By KEGG analysis, it was demonstrated that *Notch signaling* is
390up-regulated in the OV from fed females [17]. Here, we find that transcripts involved in Notch
391developmental functions, such as Fridge, presenilin enhancer 2 (PEN-2) and presenilin-1, are up-
392regulated in OV_FC (Fig 4B and S2 Table). Mastermind is an essential nuclear factor that
393supports the activity of Notch [46]. In OV_FC of *R. prolixus*, *mastermind* transcriptional factor
394is up-regulated, as well as Bx42 (Fig 4B and S2 Table), an essential factor which through Notch
395is involved in the formation of different tissues during embryogenesis [47]. In *Blattella*
396*germanica*, it was demonstrated that Notch is important in maintaining the proliferative and non-
397apoptotic state of follicular cells, as well as in differentiation of the posterior follicular cell
398population [48]. It is likely that the up-regulation of this signaling in *R. prolixus* after a blood
399meal is related to follicular cell metabolism during egg growth.

400 During vitellogenesis, JH titres are expected to increase, since JH is one of the main
401hormones involved in Vg synthesis. In insects, the corpora allata (CA), a pair of endocrine
402glands associated with the brain, are responsible for the synthesis of this sesquiterpenoid
403hormone [41]. By KEGG analysis, two pathways related to JH, “Insect hormone biosynthesis”
404and “Terpenoid backbone biosynthesis”, are up-regulated in the FB and OV during the FC [17].
405Here, we find that the levels of enzymes responsible for the synthesis of JH, in general, display
406an up-regulation in the OV and a non-statistically significant increase in the FC with respect to
407UFC (Fig 5A and S2 Table). It is important to highlight that *R. prolixus* allatectomized
408immediately after emergence as adults, continue to make a few eggs [49]. This finding suggests
409an alternate source for JH apart from the CA, but so far, this source has not been identified. Our
410results suggest that vitellogenic FB and OVs could be capable of synthesizing JH. In addition,

71
72

73

74

insect cytochrome P450s include a group of different enzymes involved in detoxication and biosynthesis of ecdysteroids and JH [50-51]. Previously, by KEGG analysis, we reported an up-regulation of metabolism of xenobiotics by cytochrome P450 in FB_FC, possibly because of an increase in hormone synthesis or/and a detoxification after a blood meal [17]. Allatostatin-C (ASTC) is a family of peptides originally associated with the control of CA activity but now known to be pleiotropic. ASTC and its paralog, ASTCC, are very similar peptides, likely generated by gene duplication, and their receptors possibly have a common ancestor as well [52]. We find a significant up-regulation of *ASTCC* mRNA expression in OV_UFC (Fig 5A and S2 Table), but so far, there is no evidence about the specific role of this peptide on OVs.

420

Fig 5. Heat map comparing the mRNA expression levels of molecules involved in juvenile hormone signaling (A), *takeout* genes (B), and ecdysteroid signaling (C) in fat body and ovaries of female adults in different nutritional condition. The input data is the readcount value from the gene expression level analysis after normalization and is presented by means of a colour scale, in which green/yellow/red represent lowest/moderate/highest expression. DESeq was used to perform the analysis

427

JH is transported from the site of synthesis to target tissues by a haemolymph carrier protein called juvenile hormone-binding protein (JHBP). JHBP protects JH molecules from hydrolysis by esterases present in the insect haemolymph [53]. The *takeout* genes (*To*) were discovered as a circadian-regulated gene and belong to the JHBPs family [54]. The *To* genes modulate various physiological processes, such as behavioral plasticity in the migratory locust *L. migratoria* and feeding in *D. melanogaster* [55-56]. In the brown planthopper *Nilaparvata*

75

76

77

78

434 *lucens*, the *To* family of genes were reported to be regulated by JH signaling [57]. Fifteen such
435 genes were identified in the antenna of *R. prolixus* [58]. Here, we find that *To* genes have a
436 unique pattern of expression according to the tissue analyzed and feeding condition (Fig 5B and
437 S2 Table). *To1*, *To2*, *To4* and *To7* mRNA expression is highly expressed in the CNS of unfed
438 insects, suggesting that starvation could induce the expression of these genes. *To9*, *To11*, *To12*
439 and *To15* mRNA expression is significantly increased in the FB from females after a blood meal,
440 *To5*, *T12* and *T13* transcripts show a significantly increased expression in OV_FC (Fig 5B and
441 S2 Table). This is the first report of an analysis of *To* genes in different tissues involved in
442 reproduction in *R. prolixus*, providing new insights into the mechanisms involved in egg
443 formation.

444 Ecdysteroids are also critical developmental hormones involved in the regulation of
445 molting and metamorphosis. The prothoracic glands (PGs) are the major source of these
446 ecdysteroids in larvae, but PGs are absent from adult insects, where alternative sites of
447 ecdysteroid production have been described. Cardinal-Aucoin et al. [59] reported that in *R.*
448 *prolixus*, between days 3 and 4 after a blood meal, ovarian ecdysteroid content increased 4–5
449 fold to a level that was sustained for the duration of egg development. This pattern is similar to
450 that seen in the hemolymph ecdysteroid titer. Two interpretations were proposed a) the ovary
451 passively absorbs hemolymph ecdysteroids or b) the ovary produces the ecdysteroids found in
452 the hemolymph. After a blood meal, we find up-regulation of 3 enzymes involved in ecdysteroid
453 synthesis in the OV, *Shade*, *Phantom* and *26-hydroxylase*, supporting the second hypothesis (Fig
454 5C and S2 Table). Similarly, in *Tribolium castaneum*, an insect with the same type of ovaries
455 (telotrophic meroistic), 20-hydroxyecdysone (20E) and its receptors are required for ovarian

79

80

20

81
82

456growth, oocyte maturation and follicle cell differentiation [60]. Overall, this work suggests that
457the OV's in *R. prolixus* females are an alternate source for ecdysteroid synthesis.

458

459Carbohydrate analysis

460 The main blood sugar in insects is trehalose, a sugar that consists of two glycosidically
461linked glucose units. Trehalose homeostasis is controlled by trehalose-6-phosphate synthase, the
462main enzyme involved in trehalose synthesis by the FB; trehalose transporter (TRET), which has
463a particular direction of transport depending on the trehalose gradient, and trehalases, specifically
464two isoforms, soluble (TRE-1) and membrane-bound (TRE-2), involved in the conversion of
465trehalose to glucose to generate energy [61-62]. DEG analysis reveals that *trehalose-6-*
466*phosphatase synthase* and *TRET* are up-regulated in the FB during the FC (Fig 6 and S2 Table).
467It is widely accepted that the vitellogenic process is an event with high energy demands. Thus,
468trehalose synthesis and release by TRET after a blood meal are steps necessary to provide energy
469to support successful vitellogenesis as well as trehalose to be taken up by developing oocytes,
470which accumulate carbohydrates as a resource for embryogenesis [63]. Supporting this finding,
471specific *phospholipase A2-like* mRNA (RPRC008617) is up-regulated in FB_FC (Fig 7 and S2
472Table). This belongs to a group of enzymes that are involved in either the formation or release
473of trehalose from FB cells [64]. In addition, after a blood meal, we find that *trehalose-6-*
474*phosphatase synthase* is down-regulated in OV (Fig 6 and S2 Table). Therefore, the trehalose
475that is stored in the OV to promote glycogen synthesis must be incorporated from extra-ovarian
476sources. In *R. prolixus*, it has been suggested that TRE-2 in ovaries could react directly with
477trehalose in the haemolymph supporting the idea that hydrolysis of trehalose at the cellular
478surface could be an obligatory step to provide glucose for carbohydrate accumulation by oocytes

83
84

85
86

479[65]. The researchers found that trehalase activity seemed not to be regulated at the
480transcriptional level after a blood meal. In addition, here we find that *TRE-2* is up-regulated in
481OVs but in unfed females (Fig 6 and S2 Table). We hypothesize that glucose obtained by the
482breakdown of trehalose could participate in the regulation of the energy necessary (contributed
483by different tissues, including OV) to maintain overall metabolism of the insect until
484physiological conditions improve. An interesting finding from our results is that *TRET* is more
485than 6-fold up-regulated in OV of fed insects (Fig 6 and S2 Table), supporting the hypothesis
486that a direct trehalose uptake from the hemolymph by TRET is the most important process
487involved in the storage of carbohydrates in ovaries.

488

489**Fig 6. Heat map comparing the mRNA expression levels of molecules involved in trehalose**
490**metabolism in fat body and ovaries of female adults in different nutritional condition.** The
491input data is the readcount value from the gene expression level analysis after normalization and
492is presented by means of a colour scale, in which green/yellow/red represent
493lowest/moderate/highest expression. DESeq was used to perform the analysis.

494

495**Lipid analysis**

496 In insects the majority of lipid reserves are found in the FB as triacylglycerol (TAG).
497Lipids are critical to support situations of high metabolic demand, such as vitellogenesis [9].
498TAG storage is mainly the result of 2 mechanisms: a) the transfer of dietary fat from the midgut
499to the FB by lipophorin (Lp), the main lipoprotein of insects, during feeding, and b) the synthesis
500of lipids from carbohydrates reserves. The participation of lipids in oocytes growth is mainly to
501supply energy for the developing embryo [23]. As the ability of insect oocytes to obtain fatty

89

90

acids by *de novo* synthesis is very small, most of the lipids in the oocyte come from the FB via the hemolymph using Lp as transport [66]. In vitellogenesis, lipid accumulation by OV is associated with a considerable reduction in the lipid content of the FB [9]. However, after a large blood meal, the triatomines must store a vast amount of TAG to support a possible period of fasting. This reality promotes a fine balance between lipid mobilization for egg growth and lipid storage to survive starvation. Here, we demonstrate that there are different types and subtypes of enzymes involved in lipid metabolism, as reported by Gondim et al. [67], and each one seems to have a particular role according to the specific tissue and physiological condition. TAG can be synthesized by 4 different pathways: a) the monoacylglycerol (MG)-pathway; b) the glycerol-3 phosphate (G3P) pathway; (c) degradation of phospholipids; or (d) deacylation of triglyceride catalyzed by lipases [9]. In *R. prolixus*, only the G3P pathway has been reported [67]. This pathway starts with acylation of G3P, catalyzed by G3P acyl transferases (GPAT). Two GPAT, GPAT1 and GPAT4, have been described in *R. prolixus* [68]. Here, we find that the mRNA expression of *GPA1* and *GPA4* is predominantly increased in the OV with respect to the FB and only *GPAT4* is up-regulated in the OV of unfed insects (Fig 7 and S2 Table). We had expected these enzyme to be increased in the FB after a blood meal but it is important to highlight that Alvez-Bezerra et al. [68] indicated that GPAT activity is regulated by a post-translational mechanisms and not at the mRNA levels. However, other transcripts for enzymes involved with the synthesis, elongation and lipid storage, such as *insect microsomal and cytosolic fatty acid synthases (FAS1 and FAS2)*, *lipid elongases and sterol regulatory element-binding protein (SREBP)* are up-regulated in the FB after a blood meal (Fig 7 and S2 Table). These finding coincide with our previous report, where we show that both, “fatty acid biosynthesis” and “fatty acid elongation”, are KEGG pathways enriched in FB_FC [17]. Fatty acid desaturases (FAD) are

91

92

23

93

94

525essentials for *de novo* FA synthesis. In *R. prolixus* we show that 2 transcripts encoding for *FAD*
526are up-regulated in both FB and OV of fed insects (Fig 7 and S2 Table). These results suggest
527that after a blood meal, FA synthesis increases and confirms that, besides incorporation of lipids
528from hemolymph, *de novo* synthesis of FAs by the FB of *R. prolixus* occurs, as was suggested by
529Pontes et al. [69]. Therefore, FAs stored in tissues could be used to synthesize TAG,
530phospholipids or be oxidized for ATP production. For any of these pathways, FAs need to be
531activated and that is the role of acetyl CoA synthetases (ACS). In *R. prolixus*, we find different
532ACS transcripts that encode short-chain ACS, regular ACS, long-chain ACS and very long chain
533ACS. All these enzymes are present in both the FB and OV, but their expression patterns depend
534on the nutritional condition (Fig 7 and S2 Table). In general, ACS could be considered more
535important during the unfed condition, suggesting that β -oxidation is an essential pathway in
536unfed insects to promote the synthesis of ATP as an energy source (Fig 7 and S2 Table). For FA
537mobilization, lipases play a critical role to catalyze the hydrolysis of TAG molecules [9]. In this
538sense, transcripts related to lipid breakdown (*lipases*) or lipid transfer (*lipophorin receptor, LpR*)
539in general are increased in the FB of unfed insects (Fig 7 and S2 Table). Among others, we also
540find an increase (not statistically significant) of *Brummer lipase-like* and *Hormone-sensitive*
541*lipase-like* mRNA expression in FB_UFC. Interestingly, in *D. melanogaster*, Brummer lipase is
542induced in the FB during starvation by FoxO-signaling [70]. Recently we showed that Foxo
543signaling is up-regulated in FB_UFC [17]. Hormone-sensitive lipase is present in the lipid
544droplet of *D. melanogaster* and is involved in FB lipid mobilization during starvation [71]. Also,
545it was reported that in insects, the activation of lipolysis is accompanied by hydrolysis of
546phospholipids from lipid droplets, which suggests that the phospholipase enzyme could be
547required to allow access of lipases to TAGs contained in the core of the lipid droplets [72]. In

95

96

24

97

98

548this context, Brummer lipase belongs to the calcium-independent phospholipase A2 (iPLA2)
549family [9]. In *N. lugens*, a deficiency of this enzyme during vitellogenesis impairs lipid
550mobilization, negatively affecting egg production [73]. The reality that Brummer lipase mRNA
551expression show only a small increase during UFC (statistically non-significant, S2 Table) could
552be due to the fact that in *R. prolixus* this enzyme is working the same as in *N. lugens*, being
553necessary in both nutritional conditions, due to a pleiotropic effect. In addition, the lipase
554maturation factor 1 is a protein involved in the post-translational maturation of secreted
555homodimeric lipases [74]. In times of high energy demand, such as starvation, insects use TAG
556stores via the coordinated action of lipases. In our experiment, *lipase maturation factor* transcript
557expression is up-regulated in OV_UFC, as is the expression of *Hydr2 (lipase activity enzyme)*,
558among other lipases (Fig 7 and S2 Table). These findings are another indication of the fine cross-
559talk between lipid synthesis and mobilization in both nutritional conditions.

560

561**Fig 7. Heat map comparing the mRNA expression levels of molecules involved in lipid**
562**metabolism in fat body and ovaries of female adults in different nutritional condition.** The
563input data is the readcount value from the gene expression level analysis after normalization and
564is presented by means of a colour scale, in which green/yellow/red represent
565lowest/moderate/highest expression. DESeq was used to do the analysis. DESeq was used to
566perform the analysis.

567

568 Given the premise that oocytes have a low capacity to synthesize lipids *de novo*, it is
569surprising to find that *FAS2*, *FAS3* and *Acetyl CoA decarboxylase* mRNAs, which are lipogenic
570enzymes involved in *de novo* synthesis of FA, are up-regulated in OV_UFC (Fig 7 and S2

99

25

100

101

102

571Table). Recently, we reported via KEGG analysis an up-regulation of “fatty acid biosynthesis
572pathway” in OV_UFC [17]. In mosquitoes, FAS is more highly expressed in diapause-destined
573females than in non-diapausing individuals [75]. This finding suggests that in *R. prolixus*, FAS
574could be working to convert carbohydrate reserves to lipid stores for use as an energy source to
575maintain OVs under optimal physiological conditions for successful reproduction when
576nutritional conditions are adequate, such as after feeding. Massive endocytosis of YPPs in oocyte
577and intense VgR, LpR and heavy-chain clathrin synthesis are all energy-dependent processes
578[76]; for that reason, lipid reserves in pre-vitellogenic oocytes (UFC) could play a critical role in
579supporting the energetic demands of the growing oocyte at the beginning of vitellogenesis.

580 In the triatomine, *Panstrongylus megistus*, lipid transfer to the developing oocyte during
581vitellogenesis is accomplished by endocytosis of Lp (through LpR) and by the classic
582extracellular lipophorin shuttle mechanism [23]. However, Machado et al. [77] suggested that in
583*R. prolixus*, endocytosis is not a pathway involved in lipid transfer to oocytes. Conversely, our
584results demonstrate that *LpR* transcript is up-regulated in OV_FC, probably to maximize lipid
585delivery to oocytes. Moreover, in mammals, it is known that once lipid levels drop, SREBP
586induces the expression of many genes involved in lipid synthesis and uptake, including the LDL
587receptor [78]. It has been reported that SREBP controls lipid uptake and accumulation in oocytes
588from *D. melanogaster* by regulation of LpR expression [79]. In our data we find up-regulation of
589*SREBP* mRNA in OV_FC (Fig 7 and S2 Table), suggesting that this transcription factor could be
590involved in lipid accumulation by the oocytes during vitellogenesis. The difference found in *R.*
591*prolixus* [77] could be due to 3 situations: a) an increase of transcript expression not resulting in
592an increase in the mature protein; b) the difference in the time chosen for the experiments; or c)
593the use of less sensitive approaches.

103

104

105

106

594 Diacylglycerol kinase (DGK) is a family of enzymes that catalyzes the conversion of
595 diacylglycerol (DAG) to phosphatidic acid (PA). We find that *DGK* transcript expression is up-
596 regulated in OV_FC (Fig 7 and S2 Table). PA is a component of the membrane phospholipids
597 and at this stage there is a high demand for membrane synthesis, which is used for oocyte growth
598 and/or for organelles formation, such as yolk granules and lipid droplets. On the other hand, PA
599 affects numerous intracellular signaling pathways, including those regulating cell growth,
600 differentiation, and membrane trafficking. Indeed, PA can bind to mTOR and promote TOR
601 signaling [80]. This finding further supports mTOR signaling activation after a blood meal in
602 OVs of *R. prolixus* [17]. Also in insects, the requirement of an acyl-CoA synthetase long chain
603 (ACSL1) for oviposition and egg viability has been reported [81]. In our work, we find up-
604 regulation of *ACSL1* mRNA in OVs during FC (Fig 7 and S2 Table). Acyl-CoA-binding protein
605 (ACBP) are small proteins that binds acyl-CoA esters with very high affinity to protect them
606 from hydrolysis.

607 Although *ACPB-2*, *ACPB-3*, *ACPB-4* and *ACBP-5* transcripts are present in both tissues, only
608 *ACPB-3* is up-regulated in FB_FC meanwhile *ACPB-4* is up-regulated in OV_FC (Fig 7 and S2
609 Table), indicating that the involvement in TAG mobilization by ACPB is specific and unique,
610 depending on the tissue and physiological condition.

611

612 **Neuropeptides and neurohormonal signaling, and serotonin**

613 A variety of neuropeptides and neurohormones have been identified in the CNS of *R.*
614 *prolixus* [82]. FB and OV development and function are largely regulated by several hormonal
615 and nutritional signals, i.e. ILP/TOR signaling [17]. Our transcriptome analysis showed no
616 significant change in mRNA expression after blood intake in CNS. However, we made a deep

107

108

109

110

617analysis in CNS, FB and OV to explore the relative expression of transcripts related to hormonal
618signaling in both nutritional conditions. Here, we discuss neuropeptides, in addition to the amine
619serotonin, and their receptors, which show high expression in some of the tissues analyzed (for
620more details see S2 Table). All neuropeptides are synthesized as part of a larger precursor
621molecule. The selective processing of those precursors determines which peptides are finally
622released by the specific cells [83]. Here, we find 7 enzymes involved in neuropeptide processing
623and all of them are expressed in the CNS, FB and OV in both nutritional conditions (Fig 8A and
624S1 Table). The results support the contribution of FB and OV for neuropeptide production in
625both nutritional condition.

626

627**Fig 8. Heat map comparing the mRNA expression levels of molecules involved in**
628**neuropeptide processing enzymes (A) and neuropeptide signaling (B) in fat body, ovaries**
629**and CNS of female adults in different nutritional condition.** The input data is the readcount
630value from the gene expression level analysis after normalization and is presented by means of a
631colour scale, in which green/yellow/red represent lowest/moderate/highest expression. DESeq
632was used to perform the analysis.

633

634 The presence of the AKH precursor and receptor [84] in OVs suggests a role in egg
635production and/or egg-laying behaviour as has been shown in other insects [85], possibly by an
636autocrine pathway. Here, we find that *AKH* transcript expression is detected in CNS but is up-
637regulated in OV_FC (Fig 8B and S2 Table). In insects, bursicon is a heterodimeric glycoprotein
638hormone which plays a key role in melanization and cuticle hardening during development of
639insects [86]. Recently, a novel function of bursicon was reported in the stimulation of Vg

111

112

113

114

640expression in the black tiger shrimp, *Penaeus monodon* [87]. In *R. prolixus*, we find higher
641expression of the *bursicon receptor* in OVs with respect to the CNS and FB (Fig 8B and S2
642Table), suggesting a novel role for this hormone in reproductive physiology in an insect. Human
643genome screening reveals the presence of another glycoprotein hormone, consisting of the novel
644alpha (GPA2) and beta (GPB5) subunits (GPA2/GPB5) [88]. In *A. aegypti*, GPA2/GPB5
645signaling has been implicated in controlling ionic balance [89]. In addition, this signaling
646pathway could play a role in spermatogenesis and oogenesis in male and female mosquitoes,
647respectively [90]. We find an up-regulation of *GPA2/GPB5 receptor* mRNA expression in OV
648and FB during UFC, suggesting an involvement of this signaling pathway in the stage prior to
649vitellogenesis (Fig 8B and S2 Table). Also, in rats, it has been reported that GPA2/GPB5 in the
650ovary may act as a paracrine regulator in reproductive processes [91]. Our results show up-
651regulation of *GPA2* mRNA in OV_UFC and conversely, up-regulation of this transcript in
652FB_FC (Fig 8B and S2 Table). Future experiments will determine the involvement of this new
653signaling pathway in insects and its interplay with reproductive processes. Calcitonin-like
654diuretic hormones (CT/DHs) are related to the mammalian calcitonin and calcitonin gene-related
655peptide hormonal system. Here, in addition to the expression in CNS, we show a high mRNA
656expression level of *CT/DH-Rs* in OV with moderate levels in the FB (Fig 8B and S2 Table).
657Previously, in *R. prolixus*, it was suggested that CT/DH-Rs signaling may have a critical, but
658unknown, role in reproductive physiology [92].

659 *R. prolixus* genome has two paralogue genes encoding capability (CAPA) peptides,
660named *RhoprCAPA-α* and *RhoprCAPA-β* [93-94]. These genes are mainly expressed in the CNS,
661supporting our transcriptome results (Fig 8B and S2 Table). *RhoprCAPA-α* expression was also
662detected in testes from 5th instar nymphs but not from adults, suggesting a role in the maturation

115

116

117

118

663of male gonads [93]. Here, we find *CAPA-β* transcript expression is up-regulated in OV_FC.
664Future experiments using gene silencing strategies will be performed to analyse the possible
665involvement of CAPA-β peptides on oocyte maturation or egg formation.

666 Pleiotropic effects of crustacean cardioactive peptide (CCAP) in insects and crustaceans
667have been described. Previously, it was reported that CCAP is involved in the fertilization
668process in *L. migratoria* since it increases the basal tonus and frequency of spontaneous
669spermathecal contractions [95]. Our results show an up-regulation of *CCAP* mRNA expression
670in OV_FC (Fig 8B and S2 Table), suggesting an autocrine regulation but future experiments are
671required to determine the specific involvement of this signaling in *R. prolixus* reproduction.

672 Ion transport peptides (ITPs) in locusts (*Schistocerca gregaria* and *L. migratoria*) were
673identified based on their antidiuretic activity on the ileum [96-97]. Later, in *T. castaneum* it was
674suggested that ITP signaling participates in ovarian maturation and female fecundity regulation
675[98]. However, its specific role in reproductive physiology in *R. prolixus* has not yet been
676reported. Here, we found an up-regulation of *ITP receptor* mRNA in both FB and OV from fed
677insects (Fig 8B and S2 Table).

678 In insects, long neuropeptide F (LNPF) has been reported as a main player in feeding
679behaviour, metabolism and stress responses [99]. Previously, in *R. prolixus*, it was reported that
680pre-follicular cells within the germarium express the NPF receptor, as do cells located between
681developing oocytes [100]. Taking into account both findings, our results suggest that LNPF
682signaling could have a critical role in oocyte maturation more than in egg production, since we
683find an up-regulation of *LNPF receptor* mRNA in OV_UFC with respect to FC (Fig 8B and S2
684Table).

119

120

121

122

685 Neuropeptide-like precursor 1 (NPLP1) was first identified in *D. melanogaster*. In *R.*
686*prolixus* NPLP1 peptides are involved in the feeding response, providing the first clues in the
687elucidation of their function [21]. We find an up-regulation of *NPLP1* transcript expression in
688OV_UFC (Fig 8B and S2 Table). The physiological role of NPLP1 signaling in reproduction is
689currently unknown.

690 By quantitative peptidomic assays, it was reported that in *R. prolixus*, NVP-like (NVPL)
691signaling is involved in the regulation of rapid events, such as diuresis/antidiuresis, and in
692delayed events such as mating and reproduction [21]. In our transcriptome analysis, we show an
693up-regulation of *NVPL* mRNA in OV_UFC (Fig 8B and S2 Table). Gene silencing techniques
694could be implemented to evaluate the role of this peptide in reproduction.

695 Myosuppressin is a neuropeptide only found in insects and crustaceans. It has been
696demonstrated to have anti-feeding activity and to inhibit gut and oviduct contraction and
697neuropeptide secretion [101]. In the Australian crayfish *Cherax quadricarinatus*, myosuppressin
698was detected in ovaries from mature females, suggesting a potential link between myosuppressin
699and reproduction [102]. Here, we also report *myosuppressin* mRNA expression in OVs of *R.*
700*prolixus* (Fig 8B and S2 Table).

701 A corticotropin-releasing factor-like peptide acts as a diuretic hormone in *R. prolixus*
702(Rhopr-CRF/DH) [103]; however, its distribution throughout the CNS and the expression of its
703receptor in feeding-related tissues as well as the female reproductive system suggests a
704multifaceted role for the neuropeptide. Adult female *R. prolixus*, injected with Rhopr-CRF/DH
705produce and lay significantly fewer eggs [104]. In addition, in locusts, CRF/DH inhibits oocyte
706growth and reduces ecdysteroid levels [105]. Here, we find an up-regulation of *CRF/DH*

123

124

125

126

707 *receptor* mRNA in OV and FB from unfed insects (Fig 8B and S2 Table), where vitellogenesis is
708 inhibited, supporting its effects as a negative regulator of reproduction.

709 By bioinformatic predictions, Ons et al. [105] showed for the first time the existence of
710 *RYamide* in *R. prolixus*. However, the functions of this signaling in insects is currently unclear.
711 We find a high expression of *RYamide* mRNA in OVs during both nutritional condition (Fig 8B
712 and S2 Table).

713 Proctolin was the first insect neuropeptide to be sequenced and synthesized and is found
714 in a variety of arthropods, including *R. prolixus* [107], where it plays a myostimulatory role on
715 anterior midgut, hindgut, heart, and reproductive tissue [108]. In the cockroach *Blaberus*
716 *craniifer*, nanomolar quantities of proctolin induce Vg uptake [109]. Here, we find for first time
717 a high expression of *proctolin receptor* mRNA in OVs, encouraging further studies to analyze
718 the role of this signaling in the reproductive organs (Fig 8B and S2 Table).

719 Serotonin (5-hydroxytryptamine or 5-HT) is an ancient monoamine
720 neurotransmitter/neurohormone. 5-HT receptors are classified based on sequence similarities
721 with their counterparts in vertebrates [110]. In *R. prolixus*, we find that mRNA expression to 5-
722 *HT receptors* is higher in the CNS but also expressed in the OV and FB (Fig 8B and S2 Table).
723 In mosquitos, 5-HT_{2B} was reported to be a critical player in the fat body-specific serotonin
724 signaling system, governing antagonistic ILP actions [111]. It would be interesting to analyse 5-
725 HTs functional role in reproductive tissues of *R. prolixus*.

726 The transcriptome data highlights directions for future research in examining the role of
727 particular neuropeptides/amines on specific responses to processes such as ovarian maturation or
728 egg formation, extending the temporal range of transcript/protein expression of these
729 neuropeptides/amines capitalizing on gene silencing assays.

127

128

129

130

730

731A brief analysis of genes related to immunity

732 The overall achievement of insects in maintaining a stable population of individuals is
733due, in part, to their ability to recognize pathogens and eliminate them successfully using the
734immune system. The immunity of insects comprises multiple elements that work in concert and,
735in general, includes physical barriers as well as innate immune responses, which lead to a
736combination of cellular and humoral immunity [112]. In recent years, it has been shown that
737reproduction and immunity can be mutually constraining since both responses are energetically
738costly, and therefore need to be traded off. In this context, increased reproductive activity
739reduces constitutive and induced immunity across a diversity of female insects [113]. However,
740metabolic changes that occur after the acquisition of a blood meal, include the induction of
741oxidative stress [114]. Increased metabolic activity during the process of blood digestion has
742been shown to alter levels of different detoxification enzymes in mosquito, which are the same as
743these implicated in insecticide detoxification; indeed blood feeding status in mosquitos confers
744increased tolerance to insecticides [115]. Thus, it is clear that the immune system is working in
745both nutritional conditions, before a blood meal, due to the stress that is generated by starvation,
746and after a blood meal, due to the potential toxicity of the molecules ingested with the blood.
747Along with all the roles described above for FB in reproduction, the FB also responds to
748microbial infection. One important humoral response is the production of inducible antimicrobial
749peptides (AMPs), which are rapidly synthesized after microorganism invasion [116]. In *D.*
750*melanogaster*, the Toll pathway (activated by fungi and gram-positive bacteria) and the Imd
751pathway (activated by gram-negative bacteria) lead to the synthesis of AMPs, not only by a
752pathogenic challenge, but also by aging, circadian rhythms, and mating [118-120]. Interestingly,

131

132

133

134

753in *R. prolixus*, we find an up-regulation of AMPs in OV_FC (Fig 9A and S3 Table), suggesting a
754role of the vitellogenic oocytes in humoral immunity, an event that has not yet been studied in
755insects. In addition, we find different mRNAs involved with both Toll and Imd pathways which
756are up- and down-regulated in FB and OV, without revealing a characteristic expression pattern
757in any of the nutritional conditions analyzed (Fig 9B, C and S3 Table). This finding clearly
758suggests that the immune system is responding to both stimuli: to detoxification of compounds
759which enter with blood intake and/or to avoid tissue damage due to stress caused by lack of food.
760In addition, Foxo transcriptional factor could promote activation of the stress-responsive Jun-N-
761terminal kinase (JNK) pathway, which antagonizes ILP signaling in *D. melanogaster*, causing
762nuclear localization of FoxO and inducing its targets, including growth control and stress defense
763genes [120]. Recently, we demonstrated that in unfed females, FoxO factor is translocated to the
764nucleus, stimulating the insulin-sensitive pathway and modulating longevity signaling in *R.*
765*prolixus* [17]. In the current work, we find up-regulation of most of the genes involved with JNK
766signaling, mainly in OV_UFC (Fig 9D and S3 Table) possibly to overcome effects of stress and
767low nutrition.

768

769**Fig 9. Heat map comparing the mRNA expression levels of molecules involved with**
770**Antimicrobial peptides (A), Imd pathway (B), Toll signaling pathway (C) and Jak-STAT**
771**signaling pathway (D) in fat body and ovaries of female adults in different nutritional**
772**condition.** The input data is the readcount value from the gene expression level analysis after
773normalization and is presented by means of a colour scale, in which green/yellow/red represent
774lowest/moderate/highest expression. DESeq was used to perform the analysis.

775

135

136

137

138

776 In *R. prolixus*, Duox is the enzyme that generates H₂O₂ in ovarian follicles is used as a
777fuel for hardening of eggshell proteins, a process essential for the acquisition of resistance to
778water loss [121]. In accordance with those finding, we show an up-regulation of *Duox* mRNA
779expression in OV_FC (Fig 10A and S3 Table). In addition, melanization and the production of
780nitric oxide (NO) and reactive oxygen species (ROS) are effector mechanisms also activated as a
781first line of defense. Upon infection, pattern recognition receptors activate downstream serine
782protease cascades that culminate in the activation of prophenoloxidase (PPO), a precursor
783activated by proteolytic cascades to phenoloxidase for *de novo* synthesis of melanin. NO is
784highly toxic for a wide variety of pathogens and is produced by nitric oxide synthase (NOS).
785ROS are produced by conserved nicotinamide adenine dinucleotide phosphate (NADPH)
786enzymes. Here, we find up-regulation of *PPO*, *NOS* mRNA levels and increases of *NADPH*
787transcript in OV_UFC (Fig 10A and S3 Table). Also, it is interesting to see that enzymes which
788performs as antioxidant elements, such catalases, thioredoxin peroxidases and glutathione
789peroxidase have their mRNA levels up-regulated in OV and FB from fed insects (Fig 10B and S3
790Table). RNA interference (RNAi) is triggered by endogenous or invading double-stranded RNAs
791(dsRNAs) that arise from hairpin structures, transposable elements, or virus infections [122]. In
792*R. prolixus* we show that in general, there is an up-regulation of mRNA molecules involved with
793RNAi signaling in OV_UFC (Fig 10C and S3 Table). These results suggest immunological
794signaling in OV of unfed insects, possibly to prevent damage during unfavorable metabolic
795conditions.

796

797**Fig 10. Heat map comparing the mRNA expression levels of various immune regulators**
798**(A), antioxidant enzymes (B) and RNA interference signaling (C) in fat body and ovaries of**

139

140

141

142

799**female adults in different nutritional condition.** The input data is the readcount value from the
800gene expression level analysis after normalization and is presented by means of a colour scale, in
801which green/yellow/red represent lowest/moderate/highest expression. DESeq was used to
802perform the analysis.

803

804 Overall, the information on immunity in hemipterans, including Triatominae vectors
805remains incomplete and fractionated [123]. The data presented here on immunity and
806reproduction in triatomine females encouraging the development of future studies to shed light
807on the relative contribution of the immune system in successful reproductive events.

808

809**Conclusions**

810 We present here a comprehensive analysis of mRNA expression of components of
811biological processes related with feeding and reproduction. Broadly, using high-throughput
812sequencing and a comparative expression analysis we find that a blood meal taken by *R. prolixus*
813females has both unique and interacting effects on CNS, OV and FB gene expression, with
814patterns of mRNA levels that are consistent with different needs according to the nutritional
815condition. Of particular interest, we show the cross-talk between reproduction and a) lipid,
816trehalose and protein metabolism, b) neuropeptide and neurohormonal signaling, and c) the
817immune system. Overall, our findings provide an invaluable molecular resource for future novel
818investigations on different tissues related with successful reproductive events, before and after
819the appropriated stimuli (blood meal). Our data opens up avenues of translational research that
820could generate novel strategies of vector population control. This includes the identification of
821specific genes for use in symbiont-mediated RNAi; a powerful technology which provides the

143

144

145

146

822potential for biocontrol against tropical disease vectors. In *R. prolixus*, the ability to
823constitutively deliver dsRNA by supplying with recombinant symbiotic bacteria generated
824against specific target genes involved in the reproductive success (Vg), have already been tested
825in laboratory trials and is effective in dramatically reducing the fitness of *R. prolixus* [18].

826

827Acknowledgement

828This research was supported by NSERC Discovery grants to A.B.L. and I.O.

829

830Reference

- 831 1. Echeverria LE and Morillo CA. American Trypanosomiasis (Chagas Disease). Infect Dis
832 Clin North Am. 2019;33: 119-134.
- 833 2. World Health Organization. Chagas disease (also known as American trypanosomiasis).
834 Who 2020. [https://www.who.int/news-room/fact-sheets/detail/chagas-disease-\(american-](https://www.who.int/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis))
835 [trypanosomiasis\)](https://www.who.int/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis)) (accessed March 30, 2020).
- 836 3. Torrico F, Gascon J, Ortiz L, Alonso-Vega C, Pinazo MJ, Schijman A, et al. Treatment of
837 adult chronic indeterminate Chagas disease with benznidazole and three E1224 dosing
838 regimens: a proof-of-concept, randomised, placebo-controlled trial. Lancet Infect Dis.
839 2018;18: 419-430.
- 840 4. Capuani L, Bierrenbach AL, Pereira Alencar A, Mendrone A Jr, Ferreira JE, Custer B, et
841 al. Mortality among blood donors seropositive and seronegative for Chagas disease
842 (1996-2000) in São Paulo, Brazil: A death certificate linkage study. PLoS Negl Trop Dis.
843 2017;11(5):e0005542.

147

148

149

150

- 844 5. Traverso L, Lavore A, Sierra I, Palacio V, Martinez-Barnette J, Latorre-Estivalis JM, et
845 al. Comparative and functional triatomine genomics reveals reductions and expansions in
846 insecticide resistance-related gene families. PLoS Negl Trop Dis. 2017;11(2):e0005313.
- 847 6. Ansari M, Moraiet M. Ahmad S. Insecticides: Impact on the Environment and Human
848 Health. In: Malik A, Grohmann E, Akhtar R, editors. Environmental Deterioration and
849 Human Health. Springer, Dordrecht. 2014. pp. 99-123.
- 850 7. Schoofs L, De Loof A, and Van Hiel MB. Neuropeptides as regulators of behavior in
851 insects. Annu. Rev. Entomol. 2017;62: 35-52.
- 852 8. Caers J, Verlinden H, Zels S, Vandersmissen HP, Vuerinckx K, Schoofs L. More than
853 two decades of research on insect neuropeptide GPCRs: an overview. Front Endocrinol
854 (Lausanne). 2012;3: 151.
- 855 9. Arrese EL, Soulages JL. Insect fat body: energy, metabolism, and regulation. Annu Rev
856 Entomol. 2010;55: 207-225.
- 857 10. Li S, Yu X, Feng Q. Fat Body Biology in the Last Decade. Annu Rev Entomol. 2019;64:
858 315-333.
- 859 11. Roy S, Saha TT, Zou Z, Raikhel AS. Regulatory pathways controlling female insect
860 reproduction. Annu Rev Entomol. 2018;63: 489-511.
- 861 12. Noriega FG. Autogeny in Three Species of Triatominae: *Rhodnius prolixus*, *Triatoma*
862 *rubrovaria*, and *Triatoma infestans* (Hemiptera: Reduviidae). Med Entomol. 1992;29:
863 273-277.
- 864 13. Chiang RG, Chiang JA. Reproductive physiology in the blood feeding insect, *Rhodnius*
865 *prolixus*, from copulation to the control of egg production. J Insect Physiol. 2017;97: 27-
866 37.

151

152

153

154

- 867 14. Gourbière S, Dorn P, Tripet F. Genetics and evolution of triatomines: from phylogeny to
868 vector control. *Heredity*. 2012;108: 190-202.
- 869 15. Nunes-da-Fonseca R, Berni M, Tobias-Santos V, Pane A, Araujo HM. *Rhodnius*
870 *prolixus*: From classical physiology to modern developmental biology. *Genesis*.
871 2017;55(5).
- 872 16. Mesquita RD, Vionette-Amaral RJ, Lowenberger C, Rivera-Pomar R, Monteiro
873 FA, Minx P, Spieth J, et al. Genome of *Rhodnius prolixus*, an insect vector of Chagas
874 disease, reveals unique adaptations to hematophagy and parasite infection. *Proc Natl*
875 *Acad Sci*. 2015;112: 14936-14941.
- 876 17. Leyria J, Orchard I, Lange AB. Transcriptomic analysis of regulatory pathways involved
877 in female reproductive physiology of *Rhodnius prolixus* under different nutritional states.
878 *Sci Reports*. In press.
- 879 18. Whitten MMA, Facey PD, Del Sol R, Fernandez-Martinez LT, Evans MC, Mitchell JJ, et
880 al. Symbiont-mediated RNA interference in insects. *Proc R Soc B*. 2016;283: 20160042.
- 881 19. Defferrari MS, Orchard I, Lange AB. Identification of the first insulin-like peptide in the
882 disease vector *Rhodnius prolixus*: Involvement in metabolic homeostasis of lipids and
883 carbohydrates. *Insect Biochem Mol Biol*. 2016;70: 148-159.
- 884 20. Bradford MM. A rapid and sensitive method for the quantitation of micrograms
885 quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*.
886 1976;72: 248-254.
- 887 21. Sterkel M, Urlaub H, Rivera-Pomar R, Ons S. Functional proteomics of neuropeptidome
888 dynamics during the feeding process of *Rhodnius prolixus*. *J Proteome Res*. 2011;10:
889 3363-3371.

155

156

157

158

- 890 22. Leyria J, Fruttero LL, Aguirre SA, Canavoso LE. Ovarian nutritional resources during the
891 reproductive cycle of the hematophagous *Dipetalogaster maxima* (Hemiptera:
892 Reduviidae): focus on lipid metabolism. *Arch Insect Biochem Physiol.* 2014;87: 148-163.
- 893 23. Fruttero LL, Leyria J, Canavoso LE. Lipids in insect oocytes: from the storage pathways
894 to their multiple functions. *Results Probl Cell Differ.* 2017;63: 403-434.
- 895 24. Santos R, Mariano AC, Rosas-Oliveira R, Pascarelli B, Machado EA, Meyer-Fernandes
896 JR, et al. Carbohydrate accumulation and utilization by oocytes of *Rhodnius prolixus*.
897 *Arch Insect Biochem Physiol.* 2008;67: 55-62.
- 898 25. Santos R, Rosas-Oliveira R, Saraiva FB, Majerowicz D, Gondim KC. Lipid accumulation
899 and utilization by oocytes and eggs of *Rhodnius prolixus*. *Arch Insect Biochem Physiol.*
900 2011;77: 1-16.
- 901 26. Sappington TW, Raikhel AS. Molecular characteristics of insect vitellogenins and
902 vitellogenin receptors. *Insect Biochem Mol Biol.* 1998;28: 277-300.
- 903 27. Blariza MJ, Leyria J, Canavoso LE, Soria NW, García BA. Dynamics of expression of
904 two vitellogenin genes in the Chagas' disease vector *Triatoma infestans*: Analysis
905 throughout pre-vitellogenesis and vitellogenesis. *Acta Trop.* 2016;156: 100-107.
- 906 28. Wojchowski DM, Parsons P, Nordin JH, Kunkel JG. Processing of pro-vitellogenin in
907 insect fat body: a role for high-mannose oligosaccharide. *Dev Biol.* 1986;116: 422-430.
- 908 29. Melo AC, Valle D, Machado EA, Salerno AP, Paiva-Silva GO, Cunha, E., et al.
909 Synthesis of vitellogenin by the follicle cells of *Rhodnius prolixus*. *Insect Biochem Mol*
910 *Biol.* 2000;30: 549-557.
- 911 30. Tufail M, Takeda M. Molecular cloning, characterization and regulation of the cockroach
912 vitellogenin receptor during oogenesis. *Insect Mol Biol.* 2005;14: 389-341.

159

160

161

162

- 913 31. Ciudad L, Piulachs MD, Belles X. Systemic RNAi of the cockroach vitellogenin receptor
914 results in a phenotype similar to that of the *Drosophila* yolkless mutant. *FEBS Journal*.
915 2006;273: 325-335.
- 916 32. Bujo H, Yamamoto T, Hayashi K, Nimpf J, Schneider WJ. Mutant oocytic low density
917 lipoprotein receptor gene family causes atherosclerosis and female sterility. *Proc Natl*
918 *Acad Sci USA*. 1995;92: 9905-9909.
- 919 33. Davail B, Pakdel F, Bujo H, Perazzolo LM, Waclawek M, Schneider WJ, et al.
920 Evolution of oogenesis: the receptor for vitellogenin from rainbow trout. *J Lipid Res*.
921 1998;39: 1929-1937.
- 922 34. Cho KH, Raikhel AS. Organization and developmental expression of the mosquito
923 vitellogenin receptor gene. *Insect Mol Biol*. 2001;10: 465-474.
- 924 35. Oliveira PL, Kawooya JK, Ribeiro JM, Meyer T, Poorman R, et al. A heme-binding
925 protein from hemolymph and oocytes of the blood-sucking insect, *Rhodnius prolixus*.
926 Isolation and characterization. *J Biol Chem*. 1995;270: 10897-10901.
- 927 36. Swarup S, Verheyen EM. Wnt/Wingless Signaling in *Drosophila*. *Cold Spring Harb*
928 *Perspect Biol*. 2012;4(6) pii: a007930.
- 929 37. Weng SC, Shiao SH. Frizzled 2 is a key component in the regulation of TOR signaling-
930 mediated egg production in the mosquito *Aedes aegypti*. *Insect Biochem Mol Biol*.
931 2015;61: 17-24.
- 932 38. Liu P, Peng HJ, Zhu J. Juvenile hormone-activated phospholipase C pathway enhances
933 transcriptional activation by the methoprene-tolerant protein. *Proc Natl Acad Sci USA*.
934 2015;112: E1871-E1879.

163

164

165

166

- 935 39. Sartain CV, Wolfner MF. Calcium and egg activation in *Drosophila*. *Cell Calcium*.
936 2013;53: 10-15.
- 937 40. Ilenchuk TT, Davey KG. The development of responsiveness to juvenile hormone in the
938 follicle cells of *Rhodnius prolixus*. *Insect Biochem*. 1987;17: 525-529.
- 939 41. Rivera Pérez C, Clifton ME, Noriega FG, Jindra M. Juvenil hormone regulation and
940 action. In: Saleuddin S, Lange AB, Orchard I editors. *Advances in Invertebrate (Neuro)*
941 *Endocrinology: A Collection of Reviews in the Post-Genomic Era*; 2019. pp. 1-77.
- 942 42. Bettencourt BR, Hogan CC, Nimali M, Drohan BW. Inducible and constitutive heat
943 shock gene expression responds to modification of Hsp70 copy number in *Drosophila*
944 *melanogaster* but does not compensate for loss of thermotolerance in Hsp70 null flies.
945 *BMC Biol*. 2008;6: 5.
- 946 43. Kollien AH, Billingsley PF. Differential display of mRNAs associated with blood
947 feeding in the midgut of the bloodsucking bug, *Triatoma infestans*. *Parasitol Res*.
948 2002;88: 1026-1033.
- 949 44. Luo M, Li D, Wang Z, Guo W, Kang L, Zhou S. Juvenile hormone differentially
950 regulates two Grp78 genes encoding protein chaperones required for insect fat body cell
951 homeostasis and vitellogenesis. *J Biol Chem*. 2017;292: 8823-8834.
- 952 45. Bi P, Kuang S. Notch signaling as a novel regulator of metabolism. *Trends Endocrinol*
953 *Metab*. 2015;26: 248-255.
- 954 46. Kitagawa M. Notch signalling in the nucleus: roles of Mastermind-like (MAML)
955 transcriptional coactivators. *J Biochem*. 2016;159: 287-294.

167

168

169

170

- 956 47. Negeri D, Eggert H, Gienapp R, Saumweber, H. Inducible RNA interference uncovers
957 the Drosophila protein Bx42 as an essential nuclear cofactor involved in Notch signal
958 transduction. *Mech Dev.* 2002;117: 151-162.
- 959 48. Irls P, Elshaer N, Piulachs MD. The Notch pathway regulates both the proliferation and
960 differentiation of follicular cells in the panoistic ovary of *Blattella germanica*. *Open Biol.*
961 2016;6: 150197.
- 962 49. Pratt GE, Davey KG. The corpus allatum and oogenesis in *Rhodnius prolixus*. I. The
963 effects of allatectomy. *J Exp Biol.* 1972;56: 201-214.
- 964 50. Feyereisen R. Insect P450 enzymes. *Annu. Rev. Entomol.* 1999;44: 507-533.
- 965 51. Wilson TG. Resistance of *Drosophila* to toxins. *Annu. Rev. Entomol.* 2001;46: 545-571.
- 966 52. Veenstra JA. Allatostatin C. and its paralog allatostatin double C: the arthropod
967 somatostatins. *Insect Biochem Mol Biol.* 2009;39: 161-170.
- 968 53. Goodman W, Schooley DA, Gilbert LI. Specificity of the juvenile hormone binding
969 protein: the geometrical isomers of juvenile hormone I. *Proc Natl Acad Sci USA.*
970 1978;75: 185-189.
- 971 54. So WV, Sarov-Blat L, Kotarski CK, McDonald MJ, Allada R, Rosbash M. Takeout, a
972 novel *Drosophila* gene under circadian clock transcriptional regulation. *Mol Cell Biol.*
973 2000;20: 6935-6944.
- 974 55. Guo W, Wang X, Ma Z, Xue L, Han J, Yu D, et al. CSP and takeout genes modulate the
975 switch between attraction and repulsion during behavioral phase change in the migratory
976 locust. *PLoS Genet.* 2011;7(2): e1001291.
- 977 56. Meunier N, Belgacem YH, Martin JR. Regulation of feeding behaviour and locomotor
978 activity by takeout in *Drosophila*. *J Exp Biol.* 2007;210: 1424-1434.

171

172

173

174

57. Lin X, Zhang L, Jiang Y. Distinct roles of Met and interacting proteins on the expressions of takeout family genes in brown Planthopper. *Front Physiol.* 2017;8: 100.
58. Latorre-Estivalis, JM, Sterkel M, Ons S, Lorenzo MG. Transcriptomics supports local sensory regulation in the antenna of the kissing-bug *Rhodnius prolixus*. *BMC Genomics.* 2020;21: 101.
59. Cardinal-Aucoin M, Rapp N, Steel CG. Circadian regulation of hemolymph and ovarian ecdysteroids during egg development in the insect *Rhodnius prolixus* (Hemiptera). *Comp Biochem Physiol A Mol Integr Physiol.* 2013;166: 503-509.
60. Parthasarathy R, Sheng Z, Sun Z, Palli SR. Ecdysteroid regulation of ovarian growth and oocyte maturation in the red flour beetle, *Tribolium castaneum*. *Insect Biochem Mol Biol.* 2010;40: 429-239.
61. Becker A, Schlöder P, Steele JE, Wegener G. The regulation of trehalose metabolism in insects. *Experientia.* 1996;52: 433-439.
62. Shukla E, Thorat LJ, Nath BB, Gaikwad SM. Insect trehalase: physiological significance and potential applications. *Glycobiology.* 2015;25: 357-367.
63. Atella GC, Gondim KC, Machado EA, Medeiros MN, Silva-Neto MA, Masuda H. Oogenesis and egg development in triatomines: a biochemical approach. *An Acad Bras Cienc.* 2005;77: 405-430.
64. Stanley D. The non-venom insect phospholipases A2. *Biochim Biophys Acta Mol Cell Biol Lipids.* 2006;1761: 1383-1390.
65. Santos R, Alves-Bezerra M, Rosas-Oliveira R, Majerowicz D, Meyer-Fernandes JR, Gondim KC. Gene identification and enzymatic properties of a membrane-bound

175

176

177

178

- 1001 trehalase from the ovary of *Rhodnius prolixus*. *Arch Insect Biochem Physiol*. 2012;81;
- 1002 199-213. (2012).
- 1003 66. Ziegler R, Van Antwerpen R. Lipid uptake by insect oocytes. *Insect Biochem Mol Biol*.
- 1004 2006;36: 264-272.
- 1005 67. Gondim KC, Atella GC, Pontes EG, Majerowicz D. Lipid metabolism in insect disease
- 1006 vectors. *Insect Biochem Mol Biol*. 2018;101: 108-123.
- 1007 68. Alves-Bezerra M, Ramos IB, De Paula IF, Maya-Monteiro CM, Klett EL, Coleman RA,
- 1008 et al. Deficiency of glycerol-3-phosphate acyltransferase 1 decreases triacylglycerol
- 1009 storage and induces fatty acid oxidation in insect fat body. *Biochim Biophys Acta Mol*
- 1010 *Cell Biol Lipids*. 2017;1862: 324-336.
- 1011 69. Pontes EG, Leite P, Majerowicz D, Atella GC, Gondim KC. Dynamics of lipid
- 1012 accumulation by the fat body of *Rhodnius prolixus*: The involvement of lipophorin
- 1013 binding sites. *J Insect Physiol*. 2008;54: 790-797.
- 1014 70. Wang B, Moya N, Niessen S, Hoover H, Mihaylova MM, Shaw R, et al. A hormone-
- 1015 dependent module regulating energy balance. *Cell*. 2011;145: 596-606.
- 1016 71. Bi J, Xiang Y, Chen H, Liu Z, Grönke S, Kühnlein RP, Huang X. Opposite and
- 1017 redundant roles of the two *Drosophila* Perilipins in lipid mobilization. *J. Cell Sci*.
- 1018 2012;125: 3568-3577.
- 1019 72. Arrese EL1, Patel RT, Soulages JL. The main triglyceride-lipase from the insect fat body
- 1020 is an active phospholipase A(1): identification and characterization. *J Lipid Res*. 2006;47:
- 1021 2656-2667.

179

180

181
182

1022 73. Lu K, Zhou J, Chen X, Li W, Li Y, Cheng Y, et al. Deficiency of Brummer impaires
1023 lipid mobilization and JH-mediated vitellogenesis in the Brown Planthopper, *Nilaparvata*
1024 *lugens*. *Front Physiol*. 2018;9: 1535.

1025 74. Doolittle MH, Ehrhardt N, Péterfy M. Lipase maturation factor 1: structure and role in
1026 lipase folding and assembly. *Curr Opin Lipidol*. 2010;21: 198-203.

1027 75. Robich RM, Denlinger DL. Diapause in the mosquito *Culex pipiens* evokes a metabolic
1028 switch from blood feeding to sugar gluttony. *Proc Natl Acad Sci USA*. 2005;102: 5912-
1029 15917.

1030 76. Clifton ME, Noriega FG. The fate of follicles after a blood meal is dependent on
1031 previtellogenic nutrition and juvenile hormone in *Aedes aegypti*. *J Insect Physiol*.
1032 2012;58: 1007-1019.

1033 77. Machado EA, Atella GC, Gondim KC, de Souza W, Masuda H. Characterization and
1034 immunocytochemical localization of lipophorin binding sites in the oocytes of *Rhodnius*
1035 *prolixus*. *Arch Insect Biochem*. 1996;31: 185-196.

1036 78. Shao W, Espenshade PJ. Expanding roles for SREBP in metabolism. *Cell Metab*.
1037 2012;16: 414-419.

1038 79. Sieber MH, Spradling AC. Steroid signaling establishes a female metabolic state and
1039 regulates SREBP to control oocyte lipid accumulation. *Curr Biol*. 2015;25: 993-1004.

1040 80. Lin YH, Chen YC, Kao TY, Lin YC, Hsu TE, Wu YC, et al. Diacylglycerol lipase
1041 regulates lifespan and oxidative stress response by inversely modulating TOR signaling
1042 in *Drosophila* and *C. elegans*. *Aging Cell*. 2014;13: 755-764.

185

186

- 1043 81. Alves-Bezerra M, Majerowicz D, Grillo LA, Tremonte H, Almeida CB, Braz GR, et al.
1044 Serotonin regulates an acyl-CoA-binding protein (ACBP) gene expression in the midgut
1045 of *Rhodnius prolixus*. *Insect Biochem Mol Biol*. 2010;40: 119-125.
- 1046 82. Ons S. Neuropeptides in the regulation of *Rhodnius prolixus* physiology. *J Insect Physiol*.
1047 2017;97: 77-92.
- 1048 83. Turner AJ. Neuropeptide processing enzymes. *Trends Neurosci*. 1984;7: 258-260.
- 1049 84. Zandawala M, Hamoudi Z, Lange AB, Orchard I. Adipokinetic hormone signalling
1050 system in the Chagas disease vector, *Rhodnius prolixus*. *Insect Mol Biol*. 2015;24: 264-
1051 276.
- 1052 85. Lorenz MW. Adipokinetic hormone inhibits the formation of energy stores and egg
1053 production in the cricket *Gryllus bimaculatus*. *Comp Biochem Physiol B Biochem Mol*
1054 *Biol*. 2003;136: 197-206.
- 1055 86. Luo CW, Dewey EM, Sudo S, Ewer J, Hsu SY, Honegger HW, et al. Bursicon, the insect
1056 cuticle-hardening hormone, is a heterodimeric cystine knot protein that activates G
1057 protein-coupled receptor LGR2. *Proc Natl Acad Sci USA*. 2005;102: 2820-2825.
- 1058 87. Sathapondecha P, Panyim S, Udomkit A. A novel function of bursicon in stimulation of
1059 vitellogenin expression in black tiger shrimp, *Penaeus monodon*. *Aquaculture*. 2015;446:
1060 80-87.
- 1061 88. Hsu SY, Nakabayashi K, Bhalla A. Evolution of glycoprotein hormone subunit genes in
1062 bilateral metazoa: identification of two novel human glycoprotein hormone subunit
1063 family genes, GPA2 and GPB5. *Mol Endocrinol*. 2002;16: 1538-1551.

187

188

189

190

- 1064 89. Paluzzi J-P, Vanderveken M, O'Donnell MJ. The heterodimeric glycoprotein hormone,
1065 GPA2/GPB5, regulates ion transport across the hindgut of the adult mosquito, *Aedes*
1066 *aegypti*. PLoS ONE. 2014;9(1): e86386.
- 1067 90. Rocco D A, Kim D, Paluzzi J-P. Immunohistochemical mapping and transcript
1068 expression of the GPA2/GPB5 receptor in tissues of the adult mosquito, *Aedes aegypti*.
1069 Cell Tissue Res. 2017;369: 313-330.
- 1070 91. Sun SC, Hsu PJ, Wu FJ, Li SH, Lu CH, Luo CW. Thyrostimulin, but not thyroid-
1071 stimulating hormone (TSH), acts as a paracrine regulator to activate the TSH receptor in
1072 mammalian ovary. J Biol Chem. 2010;285: 3758-3765.
- 1073 92. Zandawala M, Li S, Hauser F, Grimmelikhuijzen CJP, Orchard, I. Isolation and
1074 functional characterization of calcitonin-like diuretic hormone receptors in *Rhodnius*
1075 *prolixus*. PLoS ONE. 2013;8(11): e82466.
- 1076 93. Paluzzi JP, Russell WK, Nachman RJ, Orchard I. Isolation, cloning, and expression
1077 mapping of a gene encoding an antidiuretic hormone and other CAPA-related peptides in
1078 the disease vector, *Rhodnius prolixus*. Endocrinology. 2008;149: 4638-4646.
- 1079 94. Paluzzi J-P, Orchard I. A second gene encodes the anti-diuretic hormone in the insect,
1080 *Rhodnius prolixus*. Mol Cell Endocrinol. 2010;317: 53-63.
- 1081 95. da Silva R, Lange AB. The association of crustacean cardioactive peptide with the
1082 spermatheca of the African migratory locust, *Locusta migratoria*. J Insect Physiol.
1083 2006;52: 399-409.
- 1084 96. Audsley N, McIntosh C, Phillips JE. Actions of ion-transport peptide from locust corpus
1085 cardiacum on several hindgut transport processes. J Exp Biol. 1992;173: 275-288.

191

192

193

194

- 1086 97. Audsley N., Meredith J, Phillips JE. Haemolymph levels of *Schistocerca gregaria* ion
1087 transport peptide and ion transport-like peptide. *Physiol Entomol.* 2006;31: 154-163.
- 1088 98. Nagai C, Mabashi-Asazuma H, Nagasawa H, Nagata S. Identification and
1089 characterization of receptors for ion transport peptide (ITP) and ITP-like (ITPL) in the
1090 Silkworm *Bombyx mori*. *J Biol Chem.* 2014;289: 32166–32177.
- 1091 99. Nässel DR, Wegener CA. comparative review of short and long neuropeptide F signaling
1092 in invertebrates: Any similarities to vertebrate neuropeptide Y signaling? *Peptides.*
1093 2001;32: 1335-1355.
- 1094 100. Sedra L, Paluzzi J-P, Lange AB. Characterization and expression of a long
1095 neuropeptide F (NPF) receptor in the Chagas disease vector *Rhodnius prolixus*. *PLoS*
1096 *ONE* 2018;13: e0202425.
- 1097 101. Lee D, Taufique H, da Silva R, Lange AB. An unusual myosuppressin from the
1098 blood-feeding bug *Rhodnius prolixus*. *J Exp Biol.* 2012;215: 2088-2095.
- 1099 102. Nguyen TV, Cummins SF, Elizur A, Ventura T. Transcriptomic characterization
1100 and curation of candidate neuropeptides regulating reproduction in the eyestalk ganglia of
1101 the Australian crayfish, *Cherax quadricarinatus*. *Sci Reports.* 2016;6: 38658.
- 1102 103. Lee HR, Zandawala M, Lange AB, Orchard I. Isolation and characterization of
1103 the corticotropin-releasing factor-related diuretic hormone receptor in *Rhodnius prolixus*.
1104 *Cell Signal.* 2016;28: 1152-1162.
- 1105 104. Mollayeva S, Orchard I, Lange AB. The involvement of Rhopr-CRF/DH in
1106 feeding and reproduction in the blood-gorging insect *Rhodnius prolixus*. *Gen Comp*
1107 *Endocrinol.* 2018;258: 79-90.

195

196

197

198

- 1108 105. Van Wielendaele P, Dillen S, Marchal E, Badisco L, Vanden Broeck J. CRF-like
1109 diuretic hormone negatively affects both feeding and reproduction in the desert locust,
1110 *Schistocerca gregaria*. PLoS One. 2012;7(2): e31425.
- 1111 106. Ons S, Lavore A, Sterkel M, Wulff JP, Sierra I, Martinez Barnetche J, et al.
1112 Identification of G protein coupled receptors for opsins and neurohormones in *Rhodnius*
1113 *prolixus*. Genomic and transcriptomic analysis. Insect Biochem Mol Biol. 2016;69: 34-
1114 50.
- 1115 107. Lange AB, Orchard I. Proctolin in insects. In Kastin AJ, editor. Handbook of
1116 Biologically Active Peptides. New York: Academic Press, Elsevier; 2006. pp. 177-180.
- 1117 108. Orchard I, Lee DH, da Silva R, Lange AB. The proctolin gene and biological
1118 effects of proctolin in the blood-feeding bug, *Rhodnius prolixus*. Front Endocrinol
1119 (Lausanne). 2011;2: 59.
- 1120 109. Perriere C, Broussegaury P, Goudeyperriere F. Proctolin promotes vitellogenesis
1121 onset in the imaginal molt decapitated cockroach *Blaberus craniifer*. Comp Biochem
1122 Physiol A Mol Integr Physiol. 1994;108: 533-542.
- 1123 110. Vleugels R, Verlinden H, Vanden Broeck J. Serotonin, serotonin receptors and
1124 their actions in insects. Neurotransmitter. 2015;2: e314.
- 1125 111. Ling L, Raikhel AS. Serotonin signaling regulates insulin-like peptides for
1126 growth, reproduction, and metabolism in the disease vector *Aedes aegypti*. Proc Natl
1127 Acad Sci USA. 2018;115: E9822-E9831.
- 1128 112. Salcedo-Porras N, Lowenberger C. The innate immune system of kissing bugs,
1129 vectors of chagas disease. Dev Comp Immunol. 2019;98: 119-128.

199

200

201

202

- 1130 113. Schwenke RA, Lazzaro BP, Wolfner MF. Reproduction-immunity trade-offs in
1131 insects. *Annu Rev Entomol.* 2016;61: 239-256.
- 1132 114. Dana AN, Hong YS, Kern MK, Hillenmeyer ME, Harker BW, Lobo NF, et al.
1133 Gene expression patterns associated with blood-feeding in the malaria mosquito
1134 *Anopheles gambiae*. *BMC Genomics.* 2005;6: 5.
- 1135 115. Machani MG, Ochomo E, Sang D. Bonizzoni M, Zhou G, Githeko AK, et al.
1136 Influence of blood meal and age of mosquitoes on susceptibility to pyrethroids in
1137 *Anopheles gambiae* from Western Kenya. *Malar J.* 2019;18: 112.
- 1138 116. Tsakas S, Marmaras VJ. Insect immunity and its signaling: an overview. *Invert*
1139 *Surviv J.* 2010;7: 228-238.
- 1140 117. Zerofsky M, Harel E, Silverman N, Tatar M. Aging of the innate immune
1141 response in *Drosophila melanogaster*. *Aging Cell.* 2005;4: 103-108.
- 1142 118. McDonald, M. J., Rosbash, M. Microarray analysis and organization of circadian
1143 gene expression in *Drosophila*. *Cell.* 2001;107: 567-578.
- 1144 119. Peng J, Zipperlen P, Kubli E. *Drosophila* Sex-peptide stimulates female innate
1145 immune system after mating via the Toll and Imd pathways. *Curr Biol.* 2005;15: 1690-
1146 1694.
- 1147 120. Wang MC, Bohmann D, Jasper H. JNK extends life span and limits growth by
1148 antagonizing cellular and organism-wide responses to insulin signaling. *Cell.* 2005;121:
1149 115-125.
- 1150 121. Dias FA, Gandara AC, Queiroz-Barros FG, Oliveira RLL, Sorgine MHF, Braz
1151 GRC, et al. Ovarian dual oxidase (Duox) activity is essential for insect eggshell
1152 hardening and waterproofing. *J Biol Chem.* 2013;288: 35058-35067.

203

204

205

206

1153 122. Sharp PA, RNA interference--2001. *Genes Dev.* 2001;15: 485-490.

1154 123. Zumaya-Estrada FA, Martínez-Barnette J, Lavore A, Rivera-Pomar R,
1155 Rodríguez MH. Comparative genomics analysis of triatomines reveals common first line
1156 and inducible immunity-related genes and the absence of Imd canonical components
1157 among hemimetabolous arthropods. *Parasit Vectors.* 2018;11: 48.

1158

1159 **Author Contributions**

1160 J.L., A.B.L. and I.O. designed the experiments and mapped out the manuscript. J.L. performed
1161 the experiments, wrote the manuscript and prepared all the figures. A.B.L. and I.O. reviewed and
1162 contributed to the writing of the manuscript.

1163

1164 **Competing Interests**

1165 The authors declare no competing interests.

1166

1167 **Supporting information**

1168

1169 **S1 Fig. Correlation of Log₂Fold Change values in fat body (FB) and ovaries (OV) obtained**
1170 **by RNAseq and RT-qPCR data from 7 genes.** Primers used are displayed in S1 Table. The
1171 correlation coefficient between RNAseq (y-axis) and RT-qPCR (x-axis) data (log₂ fold-change)
1172 analyzed by the Pearson test were 0.9311 (a) and 0.9109 (b), with a statistical significance
1173 $p < 0.01$.

1174

209

210

1175S1 Table. Primers used by RT-qPCR assays.

1176

1177S2 Table. Details of the mRNA expression for Figs 4 and 8. Columns are: the gene name we
 1178are assigning; VectorBase code – the official gene number in the RproC3 genome assembly;
 1179OV_FC, OV_UFC, FB_FC, FB_UFC, CNS_UFC and CNS_FC show the readcount after
 1180normalization. Log₂FoldChange: log₂ (fed condition/unfed condition); *p*-adj: *p*value after
 1181normalization (the smaller the *p*-adj, the more significant the difference). Excel cell highlights in
 1182green: up-regulation in fed condition; excel cell highlights in orange: up-regulation in unfed
 1183condition. CNS_FC, central nervous system post-feeding (FC, fed condition); CNS_UFC, central
 1184nervous system before of a blood meal (UFC, unfed condition); FB_FC, fat body in FC;
 1185FB_UFC, fat body in UFC; OV_FC, ovary in FC; OV_UFC, ovary in UFC.

1186

1187S3 Table. Details of the mRNA expression of Figs 9 and 10. Columns are: the gene name we
 1188are assigning; VectorBase code – the official gene number in the RproC3 genome assembly;
 1189OV_FC, OV_UFC, FB_FC and FB_UFC are the readcount after normalization.
 1190Log₂FoldChange: log₂ (fed condition/unfed condition); *p*-adj: *p*value after normalization (the
 1191smaller the *p*-adj, the more significant the difference). Excel cell highlights in green: up-
 1192regulation in fed condition; excel cell highlights in orange: up-regulation in unfed condition.

1193

1194

1195

1196

1197

211

212

213

214

1198

1199

1200

1201

1202

1203

1204

1205

1206

1207

1208

1209

1210

1211

1212

215

216

Figure 1

Figure 2

Figure 3

Figure 4

Trehalose metabolism	VectorBase code	OV_FC	OV_UFC	FB_FC	FB_UFC
TRET	RPRC007957				
Trehalose-6-phosphate synthase	RPRC003010				
TRE-2 (membrane)	RPRC004614				
TRE-1 (soluble)	RPRC012647

Readcount values after normalization

Figure 6

Figure 7

Figure 9

Figure 10