Interaction of lipophorin with *Rhodnius prolixus* oocytes: biochemical properties and the importance of blood feeding

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Lipophorin (Lp) is the main haemolymphatic lipoprotein in insects and transports lipids between different organs. In adult females, lipophorin delivers lipids to growing oocytes. In this study, the interaction of this lipoprotein with the ovaries of Rhodnius prolixus *was characterised using an oocyte membrane preparation and purified radiolabelled Lp (^{125}I-Lp). Lp-specific binding to the oocyte membrane reached equilibrium after 40-60 min and when ^{125}I-Lp was incubated with increasing amounts of membrane protein, corresponding increases in Lp binding were observed. The specific binding of Lp to the membrane preparation was a saturable process, with a K_d of 7.1 ± 0.9 x 10^{-8} M and a maximal binding capacity of 430 ± 40 ng ^{125}I-Lp/µg of membrane protein. The binding was calcium independent and pH sensitive, reaching its maximum at pH 5.2-5.7. Suramin inhibited the binding interaction between Lp and the oocyte membranes, which was completely abolished at 0.5 mM suramin. The oocyte membrane preparation from* R. prolixus *also showed binding to Lp from* Manduca sexta. *When Lp was fluorescently labelled and injected into vitellogenic females, the level of Lp-oocyte binding was much higher in females that were fed whole blood than in those fed blood plasma.*

Key words: haematophagous insect - lipophorin receptor - ovary - oocyte - *Rhodnius prolixus*

As in all oviparous animals, oogenesis in insects is characterised by an accumulation of nutrients in the oocytes that are to be consumed during embryonic development. These substances - proteins, carbohydrates and lipids - are used as an energy source and as precursors for other molecules that will be used to create the offspring (Kawooya & Law 1988, Raikhel & Dhadialla 1992, Santos et al. 2008). The importance of lipids in insect embryogenesis is demonstrated by the composition of the insect egg, as lipids represent 30-40% of the dry weight of the egg in some species (Kawooya & Law 1988, Briegel 1990). These embryonic lipids originate mainly from haemolymph lipophorin (Lp) (Fruttero et al. 2011, Parra-Peralbo & Culi 2011, Santos et al. 2011).

Lp is a major insect lipoprotein (Chino et al. 1981, Soulages & Wells 1994, Ryan & van der Horst 2000) that transports lipids throughout the insect body. It receives lipids from the midgut or from storage organs and delivers them to utilisation sites (Arrese et al. 2001, Rodenburg & van der Horst 2005). Based on its density, this lipoprotein is classified as low-density Lp, high-density Lp (HDLp) and very HDLp (Beenakkers et al. 1988). The interaction of Lp with tissues is mediated by specific receptors and in many insects, Lp receptors have been characterised as members of the low-density lipoprotein receptor gene superfamily (Dantuma et al. 1999, Cheon et al. 2001, Ciudad et al. 2007).

In many cases, Lp functions as a reusable lipid shuttle and the delivery of lipids to the organs occurs without Lp internalisation, accumulation or degradation (van Heusden et al. 1987, Canavoso et al. 2001). In contrast, it has been shown that in some insects, cells may take up Lp particles in a situation-specific manner. For example, in the fat body of young adult *Locusta migratoria*, Lp is endocytosed and then secreted back into the haemolymph (Dantuma et al. 1997, van Hoof et al. 2005). In developing oocytes, the accumulation of Lp apoproteins may occur (Sun et al. 2000, Ciudad et al. 2007, Fruttero et al. 2011, Parra-Peralbo & Culi 2011). However, in some insects, Lp internalisation is not a pivotal event in lipid uptake by oocytes because lipid accumulation is not impaired when endocytosis is abolished. Thus, Lp seems to interact with oocytes by two mechanisms, i.e., endocytic and non-endocytic, as is the case for *Manduca sexta* and *Panstrongylus megistus* (Kawooya & Law 1988, Fruttero et al. 2011).

In *Rhodnius prolixus*, a haematophagous insect, blood meals trigger several metabolic processes, including oogenesis (Buxton 1930). In adult females, soon after feeding, the midgut transfers diacylglycerol, phospholipids and free fatty acids to the circulating Lp (HDLp) (Coelho et al. 1997); this process is most intensive dur-

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ing the first three days (Coelho et al. 1997, Grillo et al. 2007). After these first days, the participation of the fat body as a lipid supplier increases (Coelho et al. 1997). The transfer of lipids from the midgut and fat body to Lp occurs to sustain oogenesis (Atella et al. 2005); it has been reported that insect oocytes do not significantly synthesise fatty acids (Ziegler & van Antwerpen 2006). In *R. prolixus*, lipids transported by Lp to the oocytes are stored as triacylglycerol and are mobilised during embryogenesis (Santos et al. 2011). As well as in *P. megistus* (Fruttero et al. 2011) and *Drosophila melanogaster* (Parra-Peralbo & Culi 2011), lipid accumulation by growing oocytes mainly occurs without Lp endocytosis, with only a negligible amount of Lp protein present in the oocytes (Gondim et al. 1989b).

The association of Lp with its receptor has been primarily studied in the insect fat body and midgut (Dantuma et al. 1996, Gondim & Wells 2000, Pontes et al. 2002, Grillo et al. 2003, Fruttero et al. 2009) and information about the biochemical characteristics of the interaction of Lp with oocytes, an important event in lipid uptake by these cells, is scarce. Here, the binding of Lp to the membrane of *R. prolixus* oocytes was characterised. Additionally, a possible role for blood meals in Lp receptor availability was identified.

MATERIALS AND METHODS

Insects - The experimental insects were adult, mated *R. prolixus* females taken from a colony that was maintained at 28ºC and 80-90% relative humidity. The insects were fed rabbit blood at three-week intervals.

Purification of Lp from the haemolymph of R. prolixus and M. sexta - For the purification of Lp from *R. prolixus*, haemolymph was collected from adult females four-five days after consuming a blood meal, in the presence of phenylthiourea (3-13 mg/mL), 5 mM ethylenediaminetetracetic acid (EDTA), 0.15 M NaCl, 0.1 mg/mL soybean trypsin inhibitor, 100 µM leupeptin and 1 mM benzamidine (all final concentrations). The collected haemolymph was centrifuged at room temperature for 5 min at 13,000 *g* and Lp was purified from the supernatant as described previously (Golodne et al. 2001). The supernatant was diluted with phosphate buffered saline (PBS) [10 mM phosphate, 0.15 M NaCl (pH 7.4)] and adjusted to 44.5% KBr (w/v) and then 10 mL of this solution was overlaid with 10 mL of 11% KBr (w/v) in the same buffer. This material was centrifuged at 159,000 *g* in a Beckman Coulter 70 Ti rotor (Fullerton, CA, USA) at 4ºC for 20 h and the purified Lp was collected from the top of the gradient. The purified Lp was extensively dialysed against 10 mM Tris, 10 mM 3-(N-morpholino)propanesulfonic acid (MOPS) and 0.15 M NaCl (pH 6.5) and stored in liquid nitrogen until use. The degree of purification was monitored by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli 1970).

Lp (HDLp) from *M. sexta*, which was used in one set of experiments, was purified from the haemolymph as described previously (Gondim & Wells 2000). Haemolymph was collected from insects at day 4 of the fifth instar through an incision in the second pair of prolegs; the haemolymph was collected into PBS [12 mM potassium phosphate, 0.15 M NaCl (pH 6.7)] containing 2.5 mM EDTA, 5 mM glutathione, 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and 5 mM benzamidine. After centrifugation of the haemolymph for 20 min in a clinical centrifuge, the supernatant was collected for Lp purification using the same KBr gradient ultracentrifugation protocol utilised for the purification of *R. prolixus* Lp.

Lp iodination - Purified Lp from *R. prolixus* was radiolabelled with ^{125}I-sodium iodide (17.4 Ci/mg) purchased from the National Nuclear Energy Commission (São Paulo, Brazil) at 200 µCi/mg of protein using 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril (Iodo-gen™, Sigma-Aldrich Co, St. Louis, MO, USA) as previously described (Pontes et al. 2002). The specific activity of ^{125}I-Lp obtained was approximately 200,000 cpm/µg of protein.

Ovarian follicle membrane preparation - Ovaries were dissected from 250 females four days after consuming a blood meal. After washing with PBS, the tracheae that adhered to the ovaries, the ovarian sheath and the oviducts were carefully removed and the ovarioles were stored in liquid nitrogen until use. To obtain the membrane preparation, the ovarioles were homogenised (20 strokes in a Potter-Elvehjem homogeniser) in 10 mL of cold buffer [10 mM Tris, 10 mM MOPS, 0.15 M NaCl (pH 6.5)] containing 5 mM benzamidine, 1 mM PMSF, 10 µM pepstatin and 100 µM leupeptin. This homogenate was subjected to four consecutive centrifugation steps as described previously (Gondim & Wells 2000). The final pellet was resuspended in 300 µL of the above buffer (without the protease inhibitors), separated into small aliquots and stored in liquid nitrogen until use. This membrane preparation is referred to as the oocyte membrane preparation, although it was obtained from ovarioles containing the ovarian follicles. For one set of experiments, a membrane preparation was also obtained from the fat body as previously described (Pontes et al. 2002).

Protein determination - The protein concentration of the purified Lp and oocyte membrane preparations was determined according to Lowry et al. (1951) in the presence of 0.5% SDS (w/v), using bovine serum albumin (BSA) as a standard.

Binding assay - Filtration binding assays were performed as previously described (Pontes et al. 2002). Briefly, the oocyte membrane preparation was incubated for 90 min at 28ºC in the presence of ^{125}I-Lp (40 µg/mL, unless otherwise stated) in binding buffer [10 mM Tris, 10 mM MOPS, 0.15 M NaCl, 2 mM $CaCl_2$ and 2.5 mg/mL BSA (pH 6.5)]. For the determination of non-specific binding, an excess of non-radioactive Lp (1.5 mg/mL) was added. The specific binding was calculated by subtracting the non-specific binding from the total binding. For each condition, a blank was analysed in which no membrane was added. Prism 3.0 software (GraphPad Software, San Diego, CA, USA) was used to fit the curves.

Effects of pH, calcium and suramin on Lp binding - To measure the binding of ^{125}I-Lp to the oocyte membrane preparation at various pH values, a modified buffer mixture (10 mM MES, 10 mM MOPS, 10 mM Tris,

0.15 M NaCl, 2.5 mg/mL BSA and 2 mM $CaCl_2$) was used for all conditions as described previously (Gondim & Wells 2000). The pH was adjusted by the addition of either HCl or NaOH. The requirement for calcium was tested using a modified binding buffer containing 1 mM ethyleneglycol-bis(β-aminoethyl ether) N,N,N',N'-tetracetic acid (EGTA). $CaCl_2$ was added to obtain the desired Ca^{2+} concentration, according to Fabiato and Fabiato (1979) and the pH was adjusted to 6.5. To examine the effect of suramin, a polysulphated polycyclic hydrocarbon, a modified binding buffer containing different concentrations of the drug was prepared.

Labelling of Lp with Texas Red - The protein moiety of purified Lp was fluorescently labelled with the Texas Red probe (Molecular Probes, Eugene, OR, USA) according to the manufacturer's protocol with some modifications. Texas Red (70 µL of a 10 mg/mL solution in dimethylformamide) was added to 150 µL of Lp (10 mg/mL) in 0.1 M bicarbonate buffer (pH 9.0) and the mixture was incubated under gentle agitation for 18 h at 4ºC. To remove the free Texas Red, the reaction mixture was passed through two consecutive Sephadex G-50 spin columns (Sigma-Aldrich Co) (Penefsky 1977) that had been previously equilibrated with PBS. Fluorescently labelled Lp was subjected to PAGE and analysed under ultraviolet light.

Interaction of Lp with oocytes in vivo - the effect of blood meals - Adult female *R. prolixus* were fed rabbit whole blood or blood plasma in an artificial feeder (Garcia et al. 1975). On the third day after feeding, fluorescently labelled Lp (5 µL) or vehicle (PBS) was injected into the haemocoel of the females using a 10-µL syringe (Hamilton Company, Reno, NV, USA). The insects were maintained at 28ºC. After 4 h, the insects were dissected and their ovaries were isolated, washed in PBS and fixed with 4% paraformaldeyde in PBS (w/v) for 20 h at 4ºC . The follicles were isolated from the ovaries, separated by oocyte length (1 mm) and maintained in PBS containing 1% sucrose (w/v). The follicles were analysed on slides covered with 2 M n-propyl gallate in PBS:glycerol (9:1, v/v), photographed under a stereomicroscope and observed under a Zeiss LSM 310 fluorescence microscope (Carl Zeiss Inc, Jena, Germany).

RESULTS

Lp (^{125}I-Lp) binding capacities of oocyte and fat body were compared and Fig. 1 shows that the specific binding of Lp to the oocyte membrane was much higher than to the fat body membrane; which was similar to the values previously described (Pontes et al. 2002). Because the ovary membrane showed a high capacity to bind Lp, a lower membrane protein concentration (20 µg/mL) was used in the subsequent experiments. Additionally, the experimental conditions were found to be adequate because both the specific and non-specific binding of ^{125}I-Lp to the oocyte membrane preparation could be determined (Fig. 1).

As a control, the binding of ^{125}I-Lp to the different fractions produced during the oocyte membrane preparation was evaluated. As previously described for the membrane preparations from the *M. sexta* midgut and *R. prolixus* fat body and midgut (Gondim & Wells 2000, Pontes et al. 2002, Grillo et al. 2003), ^{125}I-Lp had the highest binding capacity to the final pellet, which was produced after centrifugation at 100,000 *g* (data not shown).

A time-course binding assay demonstrated that equilibrium was reached after approximately 40-60 min (Fig. 2) and other experiments were performed using an incubation time of 90 min to guarantee equilibrium conditions (Hulme & Birdsall 1992). When increasing amounts of oocyte membrane proteins were incubated with ^{125}I-Lp, Lp binding increased accordingly (Fig. 3) and at the highest membrane protein concentration used, non-specific binding accounted for approximately 15% of the specific binding. For all other experiments, 20 µg of membrane protein was used.

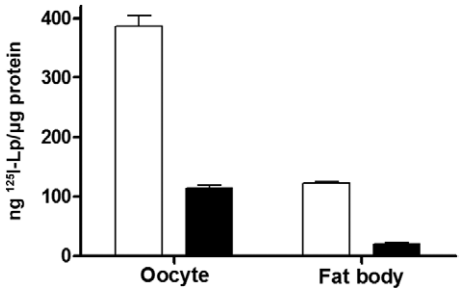

Fig. 1: comparative assay of lipophorin (Lp) binding to oocyte and fat body membranes. Specific binding of ^{125}I-Lp (40 µg/mL) to oocyte and fat body membrane preparations (40 µg protein/mL) was determined after 90 min of incubation in the presence of binding buffer (open bars). The non-specific binding (black bars) was determined in the presence of non-radioactive Lp (1.5 mg/mL). The results are expressed as ng bound ^{125}I-Lp/µg of membrane protein and they are means and standard error of the means for four determinations. Other experimental conditions were as described in Materials and Methods.

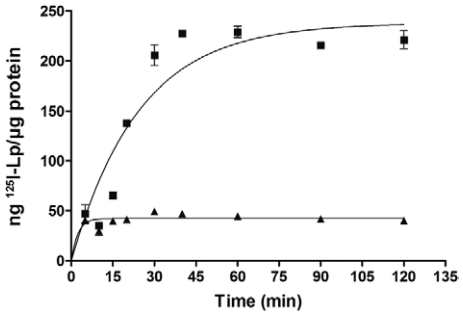

Fig. 2: time course of lipophorin (Lp) binding to oocyte membrane. ^{125}I-Lp (40 µg/mL) was incubated for different times with oocyte membrane preparation (20 µg protein/mL). The specific (■) and non-specific (▲) (in the presence of 1.5 mg/mL non-radioactive Lp) binding of ^{125}I-Lp to membranes was determined. The results are expressed as ng bound ^{125}I-Lp/µg of membrane protein. The vertical bars represent the standard error of the means for four determinations and where they are not seen they are smaller than the symbols. Specific binding curve was built by a fitting of the data using one-phase exponential association equation with Prism 3.0 software. Other experimental conditions were as described in Materials and Methods.

Lp binding was determined in the presence of increasing concentrations of ^{125}I-Lp and saturation of the binding sites was observed (Fig. 4A). In this experiment, a 3-h incubation time was used to allow equilibrium to be reached for all conditions, even in the presence of very low concentrations of ^{125}I-Lp, which is when binding takes a long time to equilibrate, as previously demonstrated (Gondim & Wells 2000, Pontes et al. 2002). Specific binding data were subjected to Scatchard analysis (Scatchard 1949) and the presence of a single type of Lp binding site was suggested (Fig. 4B). The calculated K_d was 7.1 ± 0.9 x 10^{-8} M (~22 µg/mL), with a maximal binding capacity of 430 ± 40 ng/µg of membrane protein.

Maximal specific binding occurred between pH 5.2-5.7 (Fig. 5A). The blanks, which contained no membrane, showed that no precipitation of Lp occurred in tested pH range (data not shown). Other experiments were completed at pH 6.5 because although maximal binding was not observed, the pH of the insect haemolymph commonly ranges from 6.4-7.5 (Harrison 2001) and pH 6.5 would most likely be closer to the physiological pH.

In both vertebrates and invertebrates, the binding of some ligands to their receptors is calcium dependent (Tsuchida & Wells 1990, Brown et al. 1991). The binding of ^{125}I-Lp to the *R. prolixus* oocyte membrane was not calcium dependent because the addition of 1 mM EGTA, in the absence of $CaCl_2$, did not inhibit it (Table). Moreover, the presence of different concentrations of Ca^{2+} in the incubation medium did not affect ^{125}I-Lp binding.

Suramin, a polysulphated polycyclic hydrocarbon known to inhibit the binding of lipoproteins to their receptors (Schneider et al. 1982, Tsuchida & Wells 1990, Gondim & Wells 2000, Grillo et al. 2003) prevented the binding of ^{125}I-Lp to the oocyte membrane (Fig. 5B). ^{125}I-Lp binding was abolished at 0.5 mM suramin. As previously described (Gondim & Wells 2000), the addition of more than 0.2 mM suramin causes Lp precipitation when a high concentration of the lipoprotein is present, a condition that is required to determine non-specific binding (1.5 mg/mL). Because it was only possible to measure the non-specific binding in the absence of suramin, only the total binding was determined in this assay. Lp precipitation did not occur if only ^{125}I-Lp (40 µg/mL) was used.

The capacity of the oocyte membrane to bind Lp from another insect species was also investigated using Lp from the moth *M. sexta*. As shown in Fig. 5C, unlabelled Lp from the larvae of *M. sexta* competed with ^{125}I-Lp from *R. prolixus* for the binding sites on the oocyte membrane.

To investigate the effect of diet modification on the capacity of oocytes to bind Lp, the protein moiety of Lp was fluorescently labelled with Texas Red and injected into adult females that were previously fed whole blood or blood plasma. To examine the binding of Lp to the ovaries, vitellogenic oocytes (1.0 mm in length) were analysed by fluorescence microscopy (Fig. 6). Fluorescent Lp

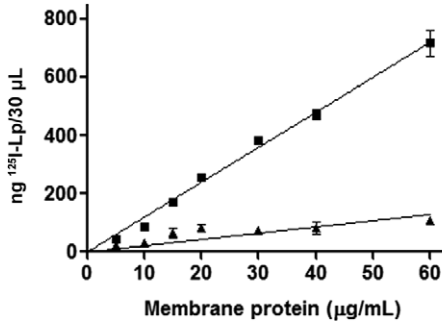

Fig. 3: effect of membrane protein concentration on the binding of lipophorin (Lp). ^{125}I-Lp (40 µg/mL) was incubated for 90 min with increasing concentrations of oocyte membrane protein and the specific (■) and non-specific (▲) (in the presence of 1.5 mg/mL non-radioactive Lp) binding of ^{125}I-Lp to the membranes was determined. The results are expressed as ng bound ^{125}I-Lp/30 µL of incubation medium. The vertical bars represent the standard error of the means for four determinations and where they are not seen they are smaller than the symbols. Other experimental conditions were as described in Materials and Methods.

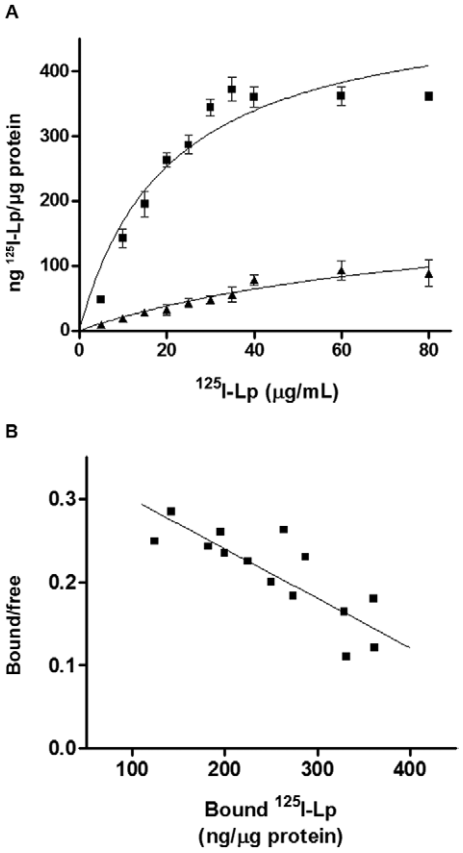

Fig. 4: determination of lipophorin (Lp) affinity for oocyte membranes. A: the oocyte membrane preparation (20 µg protein/mL) was incubated for 3 h with increasing concentrations of ^{125}I-Lp. The specific (■) and non-specific (▲) (in the presence of 1.5 mg/mL non-radioactive Lp) binding of ^{125}I-Lp to the membranes was determined. The results are expressed as ng bound ^{125}I-Lp/µg of membrane protein. The vertical bars represent the standard error of the means for four determinations and where they are not seen they are smaller than the symbols. Other experimental conditions were as described in Materials and Methods; B: Scatchard plot of the specific binding data from three different experiments. A K_d of 7.1 ± 0.9 x 10^{-8} M was calculated from three independent graphs.

bound to the oocytes in the insects fed whole blood (Fig. 6B). In contrast, the oocytes from the plasma-fed insects showed very low fluorescence, suggesting that there was little or no Lp-oocyte binding (Fig. 6D). As a control for intrinsic fluorescence, whole blood-fed insects were injected with vehicle (PBS) and showed no fluorescence (data not shown). These results indicate that diet can affect the Lp binding capacity of oocytes in *R. prolixus*. Because this interaction occurs through specific receptors, it is possible that diet has a role in their expression and/or availability at the oocyte surface.

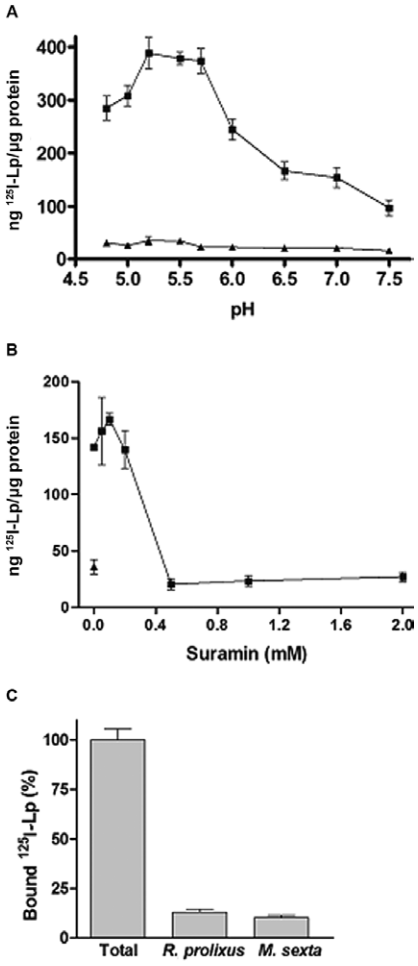

Fig. 5: effect of pH and suramin on lipophorin (Lp) binding to oocyte membranes and interaction specificity analysis. A: ^{125}I-Lp (40 µg/mL) was incubated for 90 min with oocyte membrane preparation (20 µg protein/mL) at different pH values. The specific (■) and non-specific (▲) (in the presence of 1.5 mg/mL non-radioactive Lp) binding was determined; B: the total binding of ^{125}I-Lp (40µg/mL) to the oocyte membrane preparation (20 µg protein/mL) was determined in the presence of increasing concentrations of suramin (■). The non-specific binding (▲) was measured in the presence of non-radioactive Lp (1.5 mg/mL) in the absence of suramin. In both graphics the results are expressed as ng bound ^{125}I-Lp/µg of membrane protein. The vertical bars represent the standard error of the means for four determinations and where they are not seen they are smaller than the symbols; C: oocyte membrane preparation from *Rhodnius prolixus* was incubated with *R. prolixus* ^{125}I-Lp (40 µg/mL) in the presence of an excess of non-radioactive Lp from *R. prolixus* or *Manduca sexta* (1.5 mg/mL). Total binding of ^{125}I-Lp to the membranes was obtained in the absence of non-radioactive Lp. The results are expressed as percentages of total binding and they are means and standard error of the means for four determinations. Other conditions were as described in Materials and Methods.

DISCUSSION

In this paper, we describe the interaction of Lp with its binding sites on the oocyte membrane in *R. prolixus* and show that diet may affect the capacity of oocytes to bind Lp. The specific binding of ^{125}I-Lp to the oocyte membrane was higher than to the fat body membrane.

TABLE

Effect of calcium on lipophorin (Lp) binding to oocyte membranes

Ca^{2+} (mM)	Specific binding (ng ^{125}I-Lp/µg protein)	Non-specific binding (ng ^{125}I-Lp/µg protein)
0	230 ± 10	15 ± 5
0.5	239 ±7	7.8 ± 0.9
1.0	240 ± 10	13.5 ± 0.9
2.0	230 ±1	8 ± 1

^{125}I-Lp (40 µg/mL) was incubated for 90 min with the oocyte membrane preparation (20 µg protein/mL) in the presence of 1 mM ethyleneglycol-bis(*β*-aminoethyl ether) N,N,N',N'-tetracetic acid and the required $CaCl_2$ concentrations to obtain 0, 0.5, 1.0 and 2.0 mM Ca^{2+}. The specific and non-specific (in the presence of 1.5 mg/mL non-radioactive Lp) binding was determined. The results are expressed as ng bound ^{125}I-Lp/µg of membrane protein and they are means and standard error of the means for four determinations. Other conditions were as described in Materials and Methods.

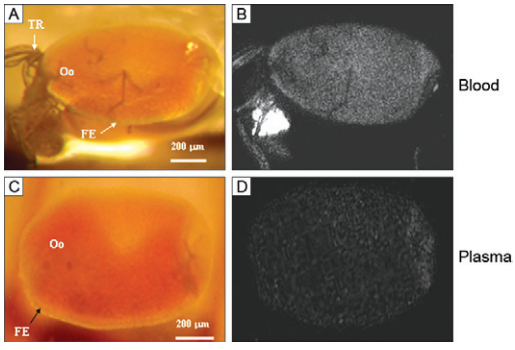

Fig. 6: effect of diet modification on lipophorin (Lp) binding to oocytes (Oo) of *Rhodnius prolixus*. Adult females were fed with whole blood or blood plasma and, three days later, injected with fluorescent Lp. After 4 h at 28ºC, insects were dissected, ovaries removed and fixed. Follicles containing 1 mm oocytes were isolated and photographed under the stereo-microscope (A, C). These follicles were also analysed by fluorescence microscopy (B, D). Images of follicles obtained from insects fed on whole blood (A, B) or blood plasma (C, D). FE: follicular epithelium; TR: tracheae. Bars = 200 µm.

This result may be due to either an increased amount of Lp receptors on the oocytes or to differences in the receptor properties between the fat body and oocytes. In *Aedes aegypti*, two distinct isoforms of Lp receptors, which are derived from a single gene, are differentially expressed in oocytes and the fat body (Seo et al. 2003). In *Bombyx mori*, four Lp receptor variants are differentially expressed; one is present in high amounts in the vitellogenic ovary, while another is more confined to the pupal fat body, though they both seem to have originated from a single gene by alternative splicing (Gopalapillai et al. 2006). In contrast, in *D. melanogaster*, two genes are transcribed into various isoforms. In *R. prolixus,* although there is no information regarding specific isoforms, only one gene encodes the Lp receptor (KC Gondim, unpublished observations). The increased binding capacity to oocytes observed here may be associated with a high rate of lipid transfer to the ovaries, which was shown to achieve its maximum between the second and fourth days after blood meal consumption (Gondim et al. 1989b, Santos et al. 2011).

The time required for ^{125}I-Lp binding to achieve equilibrium was the same as was previously observed for membranes from the fat body and midgut (Pontes et al. 2002, Grillo et al. 2003). A Scatchard plot (Scatchard 1949) indicated a single type of Lp binding site in the oocyte membrane, with a K_d of 7.1 ± 0.9 x 10^{-8} M (~22 µg/mL), which was lower than that obtained for the fat body (K_d = 2.1 ± 0.4 x 10^{-7} M) (Pontes et al. 2002), suggesting that there may be organ-specific Lp receptors. The determined K_d value indicates that the Lp receptors on the *R. prolixus* oocyte membrane are always saturated because the concentration of this lipoprotein in the haemolymph of this insect is approximately 40 mg/mL (Gondim et al. 1989a). The possibility of permanent saturation of the Lp receptors on oocytes would allow for a highly efficient lipid accumulation process, which is essential in the metabolic strategy of producing a large number of eggs in a short time. Moreover, permanent saturation seems to be a general feature for Lp receptors, as similar results were described for the fat body and midgut of *M. sexta* (Tsuchida & Wells 1990, Gondim & Wells 2000), the flight muscle of *L. migratoria* (van Antwerpen et al. 1990) and the fat body and midgut of *R. prolixus* (Pontes et al. 2002, Grillo et al. 2003). In the study that identified the presence of Lp-specific binding sites in the whole ovary of *R. prolixus* (Machado et al. 1996), the Lp concentration that was necessary to achieve a half-maximal transfer rate of phospholipids to the organ was 8 mg/mL, which is a much higher value than the K_d obtained in this study, in which isolated membranes were used. A similar pattern was also observed for the fat body of *R. prolixus* when comparing Lp binding to either a membrane preparation or the whole organ (Atella et al. 1992, Pontes et al. 2002). These differences may have occurred because the transfer of lipids between Lp and organs is a complex process that may involve other factors in addition to the receptor itself, which may affect lipid absorption by the tissue. Indeed, recent studies with *P. megistus* (Fruttero et al. 2011) and *Drosophila* (Parra-Peralbo & Culi 2011) support the idea that the main pathway of lipid uptake by insect oocytes is not endocytic. Instead, this process likely involves both Lp-specific binding sites and other oocyte surface components. Therefore, the high affinity interaction between Lp and the oocyte membrane would contribute to lipoprotein particle stabilisation on the oocyte surface, favouring lipid transfer events.

The interaction of ^{125}I-Lp with the oocyte membrane was sensitive to pH and was curiously optimal at a range below the reported haemolymph pH of various insects, which commonly varies from 6.4-7.5 (Harrison 2001). This result was similar to that described for the fat body of *L. migratoria* and the midgut of *M. sexta* (Schulz et al. 1991, Gondim & Wells 2000). In *L. migratoria*, under specific conditions, Lp is endocytosed by the fat body cells and it has been reported that the receptor does not dissociate from the lipoprotein under endosomal conditions. Instead, the ligand-receptor complex is very stable and remains intact even at pHs as low as 5.4 (Roosendaal et al. 2008). In this way, the Lp receptor on *R. prolixus* oocytes may have similar characteristics to the locust fat body receptor, as at acidic pH, the binding was very effective. Conversely, the binding of Lp to the membranes of the *R. prolixus* fat body and midgut (Pontes et al. 2002, Grillo et al. 2003) was highest at pH 6.0-7.0 (Pontes et al. 2002, Grillo et al. 2003), which may be due to differences in the receptors themselves or in the molecular characteristics of the cell membranes of each organ. For example, changes in the extracellular H^+ flux of the *R. prolixus* ovariole appear to be dynamic and could affect oogenesis (Bjornsson & Huebner 2004).

The binding of ^{125}I-Lp to the oocyte membrane was independent of calcium, similar to the fat body and midgut of *R. prolixus* (Pontes et al. 2002, Grillo et al. 2003), the midgut of *M. sexta* (Gondim & Wells 2000) and the fat body of *L. migratoria* (Dantuma et al. 1996). However, this finding differed from that observed for the fat body of *M. sexta* under the same conditions (Tsuchida & Wells 1990).

As determined for other vertebrate and insect lipoprotein receptors (Schneider et al. 1982, Dhadialla et al. 1992, Gondim & Wells 2000), suramin inhibited ^{125}I-Lp binding to the oocyte membrane, as was shown for the fat body and midgut of *R. prolixus* (Pontes et al. 2002, Grillo et al. 2003). It has been proposed that suramin, a polysulphated substance, interacts with Lp and prevents its binding to membrane receptors. This proposal is reinforced by the fact that this lipoprotein, when present at a high concentration (1.5 mg/mL), precipitated when suramin was added (Gondim & Wells 2000, Pontes et al. 2002). Moreover, in the midgut of *R. prolixus*, it has been shown that glycosaminoglycan (GAG) chondroitin-4-sulphate also inhibits Lp binding to membranes, possibly in the same way as suramin (Grillo et al. 2003). Panáková et al. (2005) showed in *Drosophila* that morphogens, i.e., secreted signalling molecules that control growth and patterning during development, can be transported by Lp. Recently, it was demonstrated that *Drosophila* Lp binds to the heparan sulphate moieties of glypicans on the imaginal disc cell surface (Eugster et al. 2007). This interaction increases the efficiency of morphogen signalling in the target cells. The ovaries

of *R. prolixus* synthesise the GAGs (primarily heparan sulphate but also chondroitin 4-sulphate) present in the ovary tissue; however, these molecules are not found inside oocytes (Costa-Filho et al. 2001). Altogether, these results raise the possibility that the interaction of Lp with its receptors on oocytes can be mediated or facilitated by GAGs. Thus, the observed suramin-induced inhibition of ^{125}I-Lp binding to the oocyte membrane could be due to the competition between suramin and ovarian GAGs.

In vertebrates, some lipoproteins can bind to receptors from different species (Biesbroeck et al. 1983, George et al. 1987). Here, we show that Lp from larval *M. sexta* was recognised by Lp receptors on *R. prolixus* oocytes. This phenomenon also occurs with the Lp receptors on *R. prolixus* fat body cells (Pontes et al. 2002). These results indicate a possible structural similarity between Lp and/or its receptors in these species, although they are from different orders. This does not seem to be a general Lp characteristic because the same lipoprotein from *M. sexta* larvae does not bind to a protein extract from the fat body of *L. migratoria* (Schulz et al. 1991).

Adult female *R. prolixus* fed blood plasma produce five times fewer eggs than insects fed whole blood (Machado et al. 1998). One of the reasons for this decrease in egg production might be a deficiency in lipid transfer to the developing oocytes. In agreement with this decrease, in the plasma-fed insects in this study, there was a decrease in the amount of Lp bound to the oocytes when compared to that in the blood-fed control females. Because the incorporation of lipids by the ovary depends on the interaction of Lp with the cell surface (Machado et al. 1996), this inhibition most likely contributed to the observed decrease in the number of laid eggs, although other factors must be considered. The observed decrease in the Lp-oocyte interaction in plasma-fed females may be due to a reduced number of binding sites on the cell surface as a result of either a decrease in receptor expression or changes in their cellular location. This result suggests that blood feeding could, in some way, regulate Lp receptors. In the same insect, the midgut membrane showed the highest capacity to bind Lp on the second day after blood meal consumption, which then decreased over the days following (Grillo et al. 2003). Accordingly, the midgut has a high capacity to transfer lipids to Lp soon after a blood meal, between days 1-3 (Coelho et al. 1997). Similarly, Lp binding to the fat body membrane also varies and at the fifth day after a blood meal, it is higher than at the second day (Pontes et al. 2008). Additionally, the capacity of the ovary to uptake lipids from Lp also changes according to the day after feeding (Gondim et al. 1989b, Santos et al. 2011). Altogether, these results suggest that the metabolic condition may be involved in the regulation of Lp receptors in *R. prolixus*. The regulation of expression of these receptors has been demonstrated in some insect species. The presence of multiple receptor isoforms has been shown and these isoforms may be expressed differentially in distinct organs and/or according to metabolic demands and hormonal control (Lee et al. 2003, Seo et al. 2003, Gopalapillai et al. 2006, Parra-Peralbo & Culi 2011). In *Galleria mellonella*, cholesterol from a lipid-rich diet up-regulates the Lp receptor (Lee et al. 2003). This result indicates that a dietary component may affect Lp receptor expression, similar to that observed in the oocytes of *R. prolixus*, where a blood meal was found to be important for Lp binding. However, the mechanisms responsible for this are unknown and deserve further investigation.

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